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ERRATA

Vol. 27, No. 2. May, 1940

page 219, line 3 *for* both by this host and *P. coccineus*
read by this host

,, ,, 10 *for* runner beans (*P. coccineus*)
read climbing varieties of *P. vulgaris*

,, ,, 15 *for* runner beans *read* climbing beans

,, ,, 19 *at end of paragraph add* Evidence regarding the susceptibility of *P. coccineus* is inconclusive.

THE CHEMICAL COMPOSITION OF THE PLANT GROWING-POINT AND ITS RELATION TO THE DAILY LIGHT EXPOSURE

By GEORGE FREDERICK SHEARD

Department of Botany, Leeds University

With 2 Text-figures

GARNER & ALLARD (1920, 1923) showed that the flowering response of many plants is markedly influenced by the daily photoperiod. Plants can be placed in one of three groups, according to their response: (*a*) "short-day" plants, which flower earlier in a short photoperiod; (*b*) "long-day" plants, which flower earlier under a long photoperiod; and (*c*) a group comprising plants which flower independently of the length of day. Other factors have been ascribed as the cause of flowering, and perhaps the most important of these is the carbohydrate/nitrogen ratio. Kraus & Kraybill (1918), working on the tomato, suggested that "the relation between the amount of vegetative and reproductive growth made by a plant, is determined by the relative amounts of carbohydrate and nitrogenous material available within it". Providing that a sufficient supply of nitrogen was available for normal growth, a high carbohydrate/nitrogen (C/N) ratio characterizes reproductive activity, whilst a low value characterizes vegetative activity. A definite type of growth would therefore be associated with the magnitude of the C/N ratio.

It is possible that the factors of photoperiod and C/N ratio are connected, since the photoperiod could have the effect of producing a C/N ratio favourable to flowering. Nightingale (1927) obtained evidence of such a relationship in the tomato. He found that an abundant supply of nitrogen and short days gave vegetative plants with a low C/N ratio, whilst long days gave fruiting plants with a high C/N ratio. The tomato is a plant which can flower in a wide range of photoperiod, but when the flowering of a short-day plant is accelerated by a short photoperiod it is difficult to understand how the favourable C/N ratio is induced. Light was thrown on this subject by Grainger (1938). From a study of long and short-day plants, his results show that in the late-flowering chrysanthemum, a typical short-day plant, there is delayed translocation of carbohydrate during the night. Reducing sugar can only be detected qualitatively a short time before dawn when the nights are long, and in the short nights of summer it cannot be detected. Artificial lengthening of the night in summer permits the detection of sugar over a longer period, and at the same time hastens flowering. Reducing sugars are detectable in long-day plants for a considerable period or exhibit other evidence of the mobility of carbohydrates. Thus the short-day plant only begins translocation after a considerable period of darkness, whilst a long-day plant has greater facilities for the translocation of carbohydrate, thus resulting in earlier flowering under long-day conditions.

Garner & Allard (1925) and Knott (1926) showed that the photoperiodic response in the case of *Cosmos* is confined to the first 2–3 cm. of the apex. These observations together with those on the chemical composition of apple spurs by Harvey (1923) and Hooker (1920) suggest that the stimulus to flower acts in and upon the apex of the plant, which is the source of all primordia, whether leaf or flower. It is, therefore, to be expected that the factors

influencing flowering would act directly or indirectly upon it, changing the production of leaf primordia into the production of flower initials. If the ratio of soluble C/N exerts a controlling influence on flowering it should appear in the growing point. The present investigation was designed to test this question experimentally.

Materials and methods

Plants were grown under controlled light conditions and the material analysed at intervals. The plants selected were *Cosmos bipinnatus* var. "Sutton's Large-Flowered Crimson Single", late-flowering chrysanthemum vars. "Golden Seal" and "Rayonanthe" and tomato var. "Kondine Red". Approximately 250, 100, 100 and 200 plants of the respective varieties were grown in large wooden boxes in compost of uniform composition. Each variety was divided into two groups, one of which received the normal day length (12–17 hr.) and the other a shortened day of 10 hr. The short days were produced by enclosing the plants in a well-ventilated light-proof hut from 6 p.m. to 8 a.m. daily.

Samples were taken every 7–10 days by removing apices 1–2 cm. long, each plant, however, only being sampled once. The fresh weight of each sample was taken and the material preserved for analysis by boiling in 95 % alcohol for 15 min. The treatment in all operations was as uniform as possible.

The "soluble" fraction of the plant material was extracted with alcohol. Analyses for nitrogen and carbohydrates were made on the extract, using Pregl's (1930) micro-Kjeldahl method for estimating nitrogen and Hanes's (1929) modification of the Hagedorn & Jensen method for sugars. Estimations of "insoluble" nitrogen were made also on the residue left after extraction.

Results

The effect of the day length on the time of flowering is shown in Table 1. Most of these results are in agreement with those of Garner & Allard (1920, 1923) and other workers. The exception is the tomato which was unaffected by the photoperiod in the present investigation, whereas Deats (1925) and Nightingale (1922) found it to be a long-day plant.

Table 1. *Phenological data*

Plant	Light conditions	Date exp. commenced	Date of bud appearance	Date of flower opening	Time to bud appearance days	Time to flowering days
Cosmos bipinnatus	Short day	5 July	20 July	7 Aug.	15	18
var. Crimson single	Long day	5 July	7 Aug.	28 Aug.	33	21
Chrysanthemum	Short day	4 July	8 Aug.	18 Oct.	35	71
var. Golden Seal	Long day	4 July	24 Sept.	—	81	—
Chrysanthemum	Short day	5 July	2 Sept.	16 Oct.	59	93
var. Rayonanthe	Long day	5 July	7 Oct.	—	94	—
Tomato	Long day	8 July	11 Aug.	—	34	—
var. Kondine Red	Short day	8 July	11 Aug.	—	34	—

Table 2. *Analytical data for* Cosmos

	Long day				Short day			
Date	Insol. N	Sol. N	Sugars	C/N	Insol. N	Sol. N	Sugars	C/N
2 July	0·677	0·074	0·80	10·8	0·677	0·074	0·80	10·8
10 July	0·742	0·095	1·73	18·2	0·656	0·095	0·98	10·3
17 July	0·719	0·038	1·89	49·5	0·754	0·076	2·52	33·3
22 July	0·765	0·070	2·48	40·3	0·644	0·065	2·74	42·1 B
29 July	0·622	0·022	2·65	119·1	0·532	0·054	2·02	37·1
3 Aug.	0·754	0·049	2·28	46·5	0·437	0·057	3·08	54·0
7 Aug.	0·667	0·042	2·61	61·8 B	0·403	0·070	2·86	41·0
9 Aug.	—	—	—	—	0·402	0·087	3·33	38·1
13 Aug.	—	—	—	—	0·335	0·073	2·37	32·8
18 Aug.	0·434	0·051	2·02	39·6	0·494	0·079	2·83	36·0
28 Aug.	0·382	0·070	2·43	33·2	0·480	0·101	2·54	25·2
14 Sept.	0·348	0·041	2·12	51·4	0·907	0·071	2·36	33·1

For explanation see under Table 5.

TABLE 3. *Analytical data for tomato*

	Long day				Short day			
Date	Insol. N	Sol. N	Sugars	C/N	Insol. N	Sol. N	Sugars	C/N
7 July	0·713	0·094	1·47	15·6	0·713	0·094	1·47	15·6
14 July	0·621	0·124	1·10	8·9	0·535	0·101	1·27	12·5
21 July	0·559	0·081	1·30	16·2	0·732	0·115	1·62	14·1
29 July	0·834	0·103	2·19	21·3	0·762	0·127	1·58	12·5
5 Aug.	0·843	0·086	1·61	16·1	0·862	0·109	1·33	12·2
11 Aug.	1·009	0·131	1·70	16·6B	0·845	0·092	1·13	12·3B
20 Aug.	1·079	0·136	2·89	21·3	0·867	0·119	2·31	19·5
28 Aug.	0·853	0·099	3·65	37·0	0·568	0·121	3·57	30·0

For explanation see under Table 5.

TABLE 4. *Analytical data for chrysanthemum "Rayonanthe"*

	Long day				Short day			
Date	Insol. N	Sol. N	Sugars	C/N	Insol. N	Sol. N	Sugars	C/N
14 July	0·795	0·110	2·99	29·1	0·790	0·105	3·00	28·5
28 July	0·692	0·086	2·79	32·5	0·699	0·099	3·12	31·6
9 Aug.	0·813	0·145	3·67	25·3	0·797	0·108	4·48	41·4
19 Aug.	0·805	0·187	2·68	14·3	0·834	0·118	3·10	26·2
3 Sept.	0·758	0·134	3·71	27·7	0·726	0·153	5·58	36·4B
15 Sept.	—	—	—	—	0·682	0·119	3·82	32·1
27 Sept.	0·684	0·096	2·82	29·3	0·662	0·084	3·04	36·2
7 Oct.	0·862	0·233	3·92	16·8B	0·729	0·074	2·52	37·2
16 Oct.	0·921	0·205	4·43	21·6	0·467	0·055	3·02	54·3

For explanation see under Table 5.

TABLE 5. *Analytical data for chrysanthemum "Golden Seal"*

	Long day				Short day			
Date	Insol. N	Sol. N	Sugars	C/N	Insol. N	Sol. N	Sugars	C/N
7 July	0·750	0·110	5·62	52·7	0·750	0·110	5·62	52·7
14 July	0·871	0·111	4·00	36·1	0·782	0·095	3·23	34·1
28 July	0·787	0·091	2·80	31·0	0·719	0·087	2·70	30·9
9 Aug.	0·872	0·081	3·92	48·3	0·838	0·124	3·69	34·4B
19 Aug.	0·831	0·077	3·53	45·8	0·762	0·108	4·10	38·2
2 Sept.	0·641	0·118	3·60	30·4	0·992	0·117	2·80	24·0
15 Sept.	0·716	0·097	3·74	38·5	0·700	0·099	2·44	24·6
27 Sept.	0·814	0·159	5·42	34·1B	0·708	0·085	2·00	23·6
7 Oct.	0·888	0·150	4·15	27·6	0·679	0·078	1·81	23·2
16 Oct.	1·173	0·198	5·78	29·2	0·657	0·102	2·32	21·6

Tables 2–5. Insol. N. = protein nitrogen. Sol. N. = soluble nitrogen. C/N = ratio of sugars to soluble nitrogen. "B" indicates the point at which flower buds were visible. Figures are given as percentage of the fresh weight, but form changes are the same when the results are expressed on a dry-weight basis. Sugar figures represent reducing sugars only, as the reducing power of the extract was in no case increased on hydrolysis.

The results of the chemical analyses are set forth in Tables 2–5 together with the ratio of soluble C/N. The latter is further shown graphically in Fig. 1.

Discussion

The results of the present investigation show that in no case was the transition from vegetative to flowering organization associated with a high or significant change in the ratio of soluble carbohydrate/soluble nitrogen in the apex. The seeming relation shown by *Cosmos* and chrysanthemum "Rayonanthe", respectively, in long days is not deemed to be

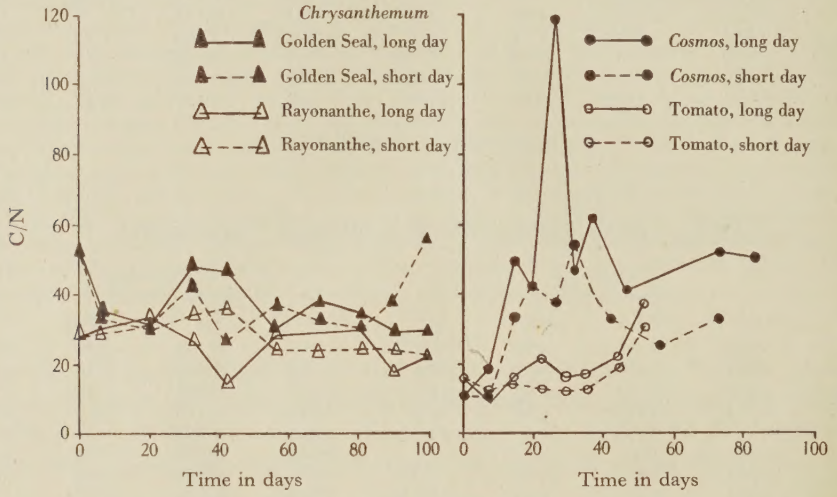

Fig. 1. Cycle of changes in the C/N ratio in chrysanthemum, *Cosmos* and tomato.

significant, as it did not hold for the short-day series or for the other plants examined. These formed their flower buds as many days after a minimum C/N ratio as the long-day set did after a maximum C/N ratio.

There are indications that flower-bud appearance may be associated with high carbohydrate. The high sugar concentration in three of the plants examined, namely, *Cosmos* and the two varieties of chrysanthemum, occurs at or about bud appearance. Tomato provided an exception, having only a relatively low sugar content at this period, but the indefinite mode of flowering of this plant makes it difficult to determine the exact time of transition from vegetative to flowering organization.

The growing points of *Cosmos* and tomato show definite cycles in the protein content of the apex. These cycles are affected by the length of days. Fig. 2 shows the quantitative changes of such a cycle. In the case of *Cosmos* the cycle of changes is the same in form and magnitude both under long and short days. Fig. 2 shows that if the long-day graph is moved 15–18 days to the left, the two curves fit very closely, the data of Table 1 providing further evidence of this similarity.

The general cycle of changes in *Cosmos* and tomato is the same whether the results are expressed on a fresh- or a dry-weight basis, showing that the changes are absolute and not due to any variation in water content. Furthermore, these results are substantiated by preliminary experiments carried out in 1936–7. Similar cyclical changes have been reported earlier by Billimoria (1936) for *Helianthus tuberosus* and by Chibnall (1922) for *Phaseolus*

multiflorus. Both investigators obtained form changes in protein nitrogen which approximate well with those obtained in the present experiments.

Such cycles are absent from both varieties of chrysanthemum examined, and it is possible that they have no major relationship with flowering. It may be, however, that in the case of *Cosmos* and tomato they are linked with the general problem of phasic development and the

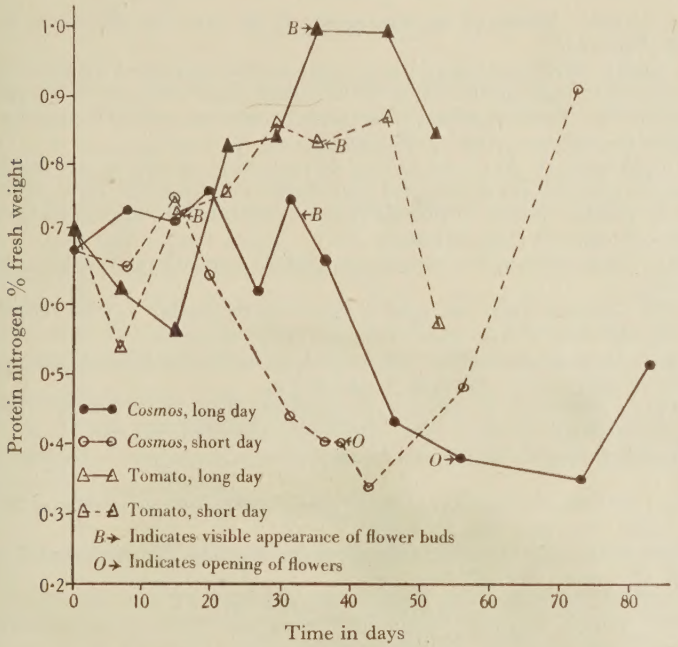

Fig. 2. Cycle of changes in protein nitrogen in *Cosmos* and tomato.

thesis of Lysenko (see Maximov, 1935) that "the entire process of development of an annual plant consists of a series of changes which proceed in a strict sequence and each of which requires for its completion differing external conditions". This cycle in protein nitrogen may be an expression of the whole or part of this sequence, the photoperiod, in the case of those plants which respond to it, acting so as to accelerate or retard the cycle.

Summary

1. No general causal relationship can be found between the onset of flowering under a favourable photoperiod and a suitable ratio of soluble C/N in the apices of chrysanthemum, *Cosmos* and tomato.

2. Flower-bud appearance in *Cosmos* and late-flowering chrysanthemum may be associated with a high sugar content.

3. *Cosmos* and tomato provide clear indications of a cycle in protein nitrogen which is affected by the photoperiod when the latter influences the time of flowering.

Thanks are due to Prof. J. H. Priestley of Leeds University, in whose department this work was carried out; to Prof. W. H. Pearsall of Sheffield University, under whose direction the investigation was made; and to Dr J. Grainger of the Tolson Memorial Museum, Huddersfield, for facilities in the growing of the material and much helpful criticism.

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METABOLISM AND FLOWERING

By JOHN GRAINGER

Tolson Memorial Museum, Ravensknowle, Huddersfield

With 9 Text-figures

Two earlier papers have established (1) that the late-flowering chrysanthemum, a typical "short-day" species, flowered in autumn because of a disability in nocturnal translocation which limited the amount of carbohydrate available at the growing-point during the short nights of summer (Grainger, 1938), and (2) that many plants which flower in spring and early summer actually begin the formation of their flower initials in the autumn and winter of the previous year (Grainger, 1939). Such plants might conceivably be "short-day" kinds, since the actual transformation from vegetative to floral organization of the growing-point was accomplished when the days were short. It therefore appears necessary to investigate their relative diurnal carbohydrate metabolism, in order to discover whether it has any causal relationship with the time of actual flower initiation, as was demonstrated for the late-flowering chrysanthemum. The present paper describes the results of such an investigation, and also of certain contributory studies as to the converse effect of flowering upon metabolism, the relative amount of energy used in flowering, and the possible inadequacy of nutrition in very floriferous plants.

Diurnal carbohydrate metabolism in relation to the time of flowering

Methods. A representative series of eleven plant species covering flower initiation throughout the whole year was studied. Their times of floral initiation were determined by dissection of representative buds at various times (see Grainger, 1939). Methods of analysis for carbohydrate contents, and the different interpretations of quantitative and qualitative estimations, have also been described (Grainger, 1938).

Results. The times of flower initiation and of flowering of the selected plants are shown in Table 1, and the calibre of their carbohydrate metabolism in Table 2 and Figs. 1–5. Water content, soluble matter, reducing sugars, non-reducing sugars and total carbohydrate were determined for all the species mentioned, but the only fraction which yielded data significant for the present purpose was that of total carbohydrate, and Figs. 1–5 include this information only for each species. Table 2 shows a complete set of estimations for *Forsythia suspensa*, where young leaves were also compared with old ones. The results indicate that there is a considerable variation between the behaviour of old and young leaves, and emphasize the necessity for measuring the diurnal carbohydrate metabolism upon samples of young leaves (compare Grainger, 1938).

All the plant species except *Salix caprea*, *Centaurea nigra* and *Saxifraga tridactylites* show a considerable fall in total carbohydrate content during the hours of darkness. This is the surest evidence of carbohydrate mobility. The three species show little variation in the content of total carbohydrate throughout the whole 24 hr., under the same climatic conditions which allowed a nocturnal diminution in the other species. Young leaves were used in every

case, so it is not the effect of old leaves demonstrated for *Forsythia*. The results of qualitative determinations of the diurnal carbohydrate metabolism of *Saxifraga tridactylites* have already been published (Grainger, 1938), and for *Centaurea nigra* and *Salix caprea* are shown in Fig. 6. These demonstrate that reducing sugars are present throughout the whole daily period, and thus supply alternative evidence of mobility of their carbohydrate. It is probable that this state of affairs signifies complete mobility of this kind of food.

TABLE 1. *Times of formation of flower initials and of flowering*

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Betula alba L., male	.	.	*30	.	−29	−20	+10	+26	+30	+	+	+
Betula alba L., female	.	.	.	.	.	.	.	+26	+30	+	+	+
Centaurea nigra L.	.	.	.	− 9	−20	+ 6	+20	*13	.	.	.	.
Chrysanthemum, early flowering	.	.	.	.	−16	−15 +27	+29	*29	.	.	.	.
Chrysanthemum, late-flowering	.	.	.	.	−16	−25	−24	+ 1	+23	*25	.	.
Crataegus Oxyacantha L.	+	+10	+ 2	+	* 3	.	− 9	−20	+23	+	+	+ 3
Forsythia suspensa Hort.	+	+	* 1	.	.	.	− 9	+20	+25	+	+24	+
Prunus spinosa L.	+	+11	+	*13	.	.	−15	−19	+23	+	+	+ 3
Rosa canina L.	.	.	−12	+24	+ 7	*25	.	.	..	−14	.	− 3
Salix caprea L., male	+	+	*11	.	−20	−15	+ 9	+	+12	+	+	+
Salix caprea L., female	+	+	*11	.	−20	−17	+15	+	+25	+	+	+
Saxifraga tridactylites Hort.	+ 9	+20	+ 1	+15	*11	−14	.	.	−20	−10 +29	+16	+14
Scabiosa succisa L.	.	.	.	− 9	−28	+22	+20	*13	.	.	.	.
Sedum spectabile Hort.	.	.	.	− 3	+20	+	*10	.	.	−16	−14	.
Sisymbrium alliaria Scop.	+17	+22	+21	*24	.	− 6	−20	− 1	.	−29	.	− 3

The numbers represent the days of the months under which they occur. + and − refer to flower initials, and * denotes the time of first flowering or maximum flowering. Thus "−29" under the month of May means that no flower initial could be found on 29 May; "+10" under July indicates that flower initials were observed on 10 July, and "*13" under August means that the species was first observed to flower on 13 Aug.

Each result is the product of a number of dissections, usually not less than six, and if one dissection yields a flower bud, the result is shown as + in the table. Plus signs without numbers are included with some species to indicate continuity. Thus in *Prunus spinosa* flower initials which can be found on 23 Sept. would ultimately form the complete flower which emerges on 13 April of the following year.

TABLE 2. *Carbohydrate contents of* Forsythia suspensa, 8–9 *Aug.* 1936

	Water content, % of fresh weight	Insoluble solids, % of dry weight	Reducing sugars, % of dry weight	Non-reducing sugars, % of dry weight	Insoluble carbohydrate, % of dry weight	Total carbohydrate, % of dry weight
			Young leaves			
8 Aug. 1.45 p.m.	72·97	66·19	26·05	14·69	24·36	65·10
6.15 p.m.	70·83	62·08	24·36	24·45	24·94	73·75
10.15 p.m.	70·73	62·65	33·73	20·60	24·67	79·00
9 Aug. 2.00 a.m.	74·38	64·27	28·85	13·18	24·70	66·73
5.30 a.m.	73·27	66·04	30·17	3·31	27·85	61·53
9.00 a.m.	75·25	61·71	28·03	2·14	28·02	58·19
2·15 p.m.	72·83	61·30	26·26	6·96	28·28	61·50
			Older leaves			
8 Aug. 1.45 p.m.	77·86	63·00	19·67	15·76	20·42	55·75
6.15 p.m.	75·33	56·91	27·36	16·37	17·77	61·50
10.15 p.m.	75·84	58·79	30·59	12·32	15·67	58·58
9 Aug. 2·00 a.m.	75·75	65·42	21·35	24·19	20·36	65·90
5.30 a.m.	75·67	70·10	21·78	10·82	19·40	52·00
9.00 a.m.	74·75	61·32	25·72	19·85	17·73	63·25
2·15 p.m.	79·56	72·40	15·29	15·00	18·96	49·25

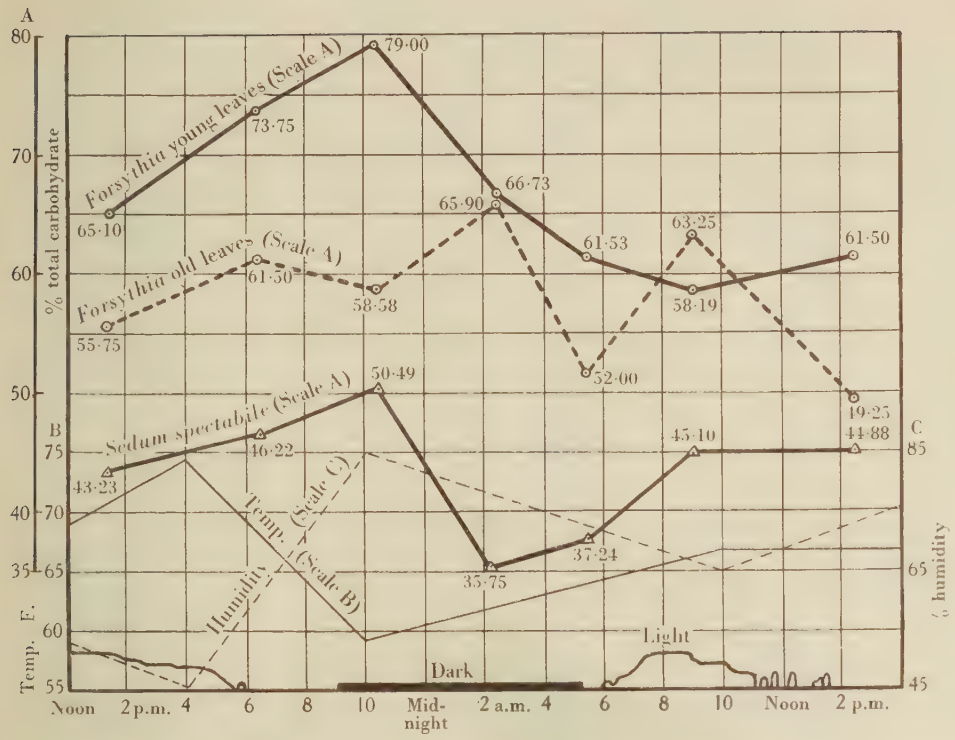

Fig. 1. Diurnal carbohydrate metabolism of *Forsythia suspensa* and *Sedum spectabile*, with meteorological data, 8–9 August 1936. The curve showing light intensity is a free transcript from a Campbell-Stokes record.

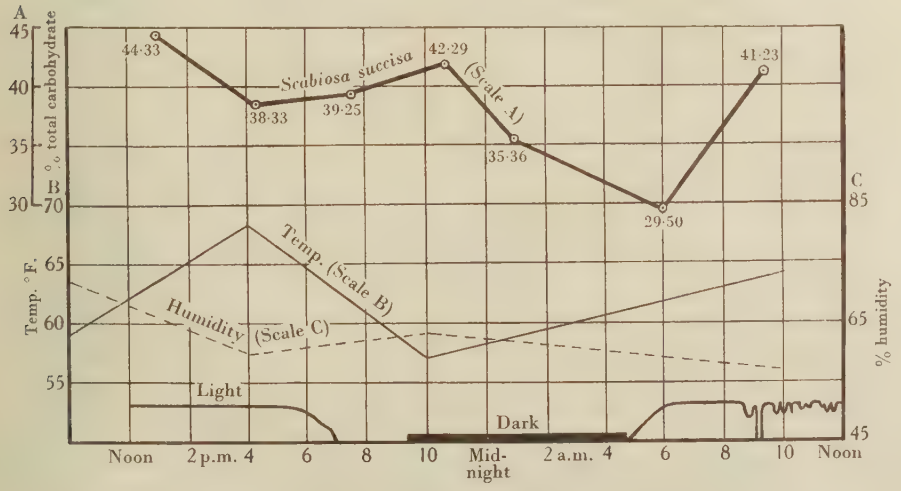

Fig. 2. Diurnal carbohydrate metabolism of *Scabiosa succisa*, with meteorological data, 26–27 June 1937. The curve showing light intensity is a free transcript from a Campbell-Stokes record.

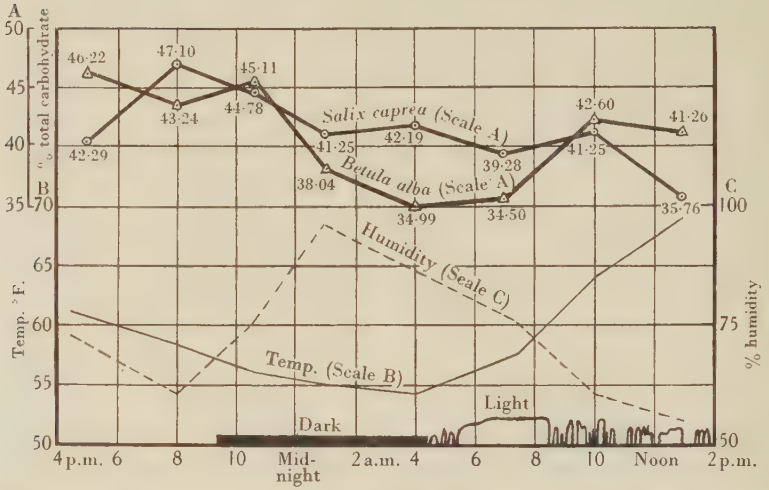

Fig. 3. Diurnal carbohydrate metabolism of *Salix caprea* and *Betula alba*, with meteorological data, 10–11 July 1937. The curve showing light intensity is a free transcript from a Campbell-Stokes record.

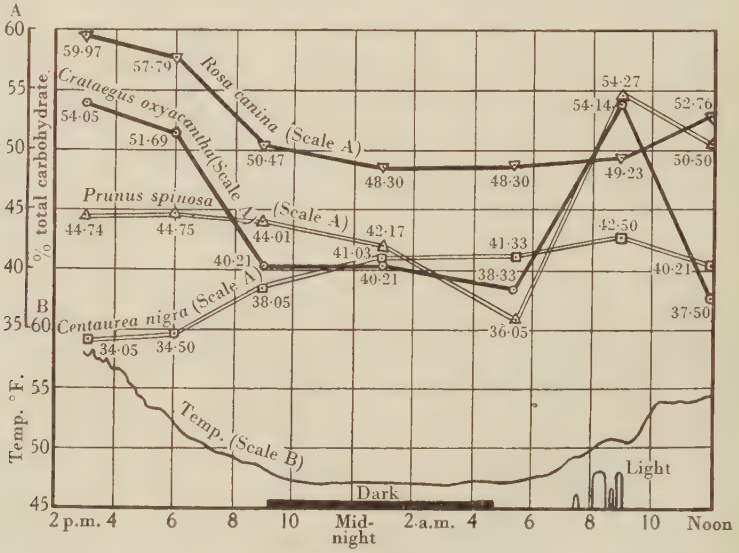

Fig. 4. Diurnal carbohydrate metabolism of *Rosa canina*, *Crataegus Oxyacantha*, *Prunus spinosa* and *Centaurea nigra*, with meteorological data, 11–12 June 1938. The temperature curve is from a thermograph record, and the curve showing light intensity is a free transcript from a Campbell-Stokes record.

All the species enumerated in Table 1 are, therefore, shown to possess mobile carbohydrate. *Saxifraga tridactylites* and *Sisymbrium alliaria* begin to make their flower initials in the short days of October and January respectively. These results, therefore, demonstrate that the delayed nocturnal translocation described for the late-flowering chrysanthemum is not an invariable cause of short-day flowering.

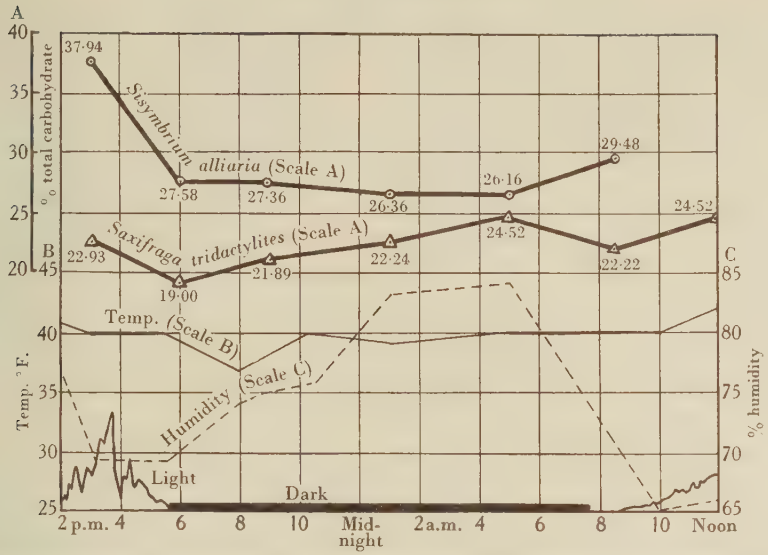

Fig. 5. Diurnal carbohydrate metabolism of *Sisymbrium alliaria* and *Saxifraga tridactylites*, with meteorological data, 28–29 January 1939. The curve showing light intensity is from an electrometrical record, giving variations of light too weak to mark the Campbell-Stokes card.

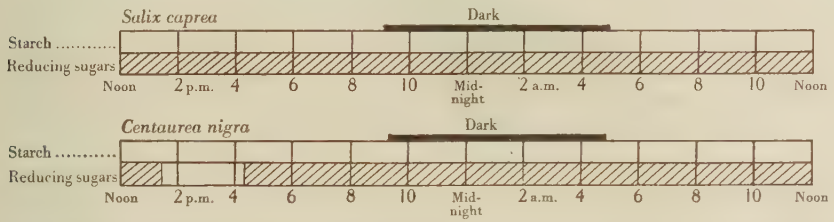
Salix caprea
Dark
Starch
Reducing sugars
Noon 2 p.m. 4 6 8 10 Mid-night 2 a.m. 4 6 8 10 Noon
Centaurea nigra
Dark
Starch
Reducing sugars
Noon 2 p.m. 4 6 8 10 Mid-night 2 a.m. 4 6 8 10 Noon

Fig. 6. Qualitative diurnal carbohydrate metabolism of *Salix caprea*, 10–11 July 1937, and of *Centaurea nigra*, 11–12 June 1938.

The relative metabolic energy involved in flowering

Since flowering can be initiated in some plants, e.g. Coltsfoot (Grainger, 1939), in the dormant season, it does not seem that much energy is required for the actual change from vegetative to floral organization of the growing-point. Estimations of the relative amounts of dry matter in the flowers or fruits and in the whole plant have been made in order to discover what proportion a number of species of diverse character expend upon the reproductive phase.

Methods. Whole plants or clumps of plants were lifted, and the soil carefully washed away from their roots. The shoots were cut off at ground level, and then the leaves and flowers or fruits were removed at their junctions with the stem. Methods of drying and of carbohydrate analysis were described by Grainger (1938); total nitrogen was estimated by a modified micro-method (Pregl, 1930), and measurements of the relative intensity of respiration were made by the standard method of CO_2 absorption in a Pettenkofer tube.

Results. Table 3 shows the results for six plants of different habit: flowering does not in general account for more than a small percentage of dry weight, and fruiting usually accounts for a greater proportion.

TABLE 3. *Distribution of dry weight in reproductive and non-reproductive plant parts*

	Date	Condition	Total dry weight of plant in g.	Dry weight of reproductive parts in g.	% reproductive dry weight of total dry weight
Anemone nemorosa L.	7 June 1938	Fruiting	17·83	0·047	0·32
Epilobium angustifolium L.	2 Aug. 1938	Flowering	15·35	1·0	6·51
Epilobium angustifolium L.	24 Sept. 1939	Fruiting	52·2	7·27	14·70
Lupinus polyphyllus Hort.	11 July 1939	Early fruiting	640·0	145·0	22·69
Luzula campestris DC.	11 Apr. 1939	Flowering	0·312	0·032	10·25
Lysimachia punctata Hort.	16 Sept. 1938	Flowering	55·35	2·7	4·90
Vaccinium Vitis-idaea L.	7 Apr. 1939	Flowering	38·0	0·26	0·69
Vaccinium Vitis-idaea L.	30 Oct. 1939	Fruiting	39·86	2·22	5·57

The lupin has the highest percentage of dry weight devoted to the reproductive organs of any species in Table 3. This plant, however, showed signs that the amount of available food was not always adequate for the needs of the very numerous developing flowers and fruits. This question is considered further on p. 318.

A more exact measure of the distribution of dry weight amongst the various organs of a plant was made with *Epilobium angustifolium* (Table 4). Estimations of respiration intensity of roots, stems, leaves, flowers and fruits (Table 5) were used to obtain an indication of the amounts of metabolic material which had been respired during the formation of each organ (Table 6). This was calculated upon the basis of the oxidation of hexose to carbon dioxide and water. Averages of the dry weight of each organ in the separate months from March to September were obtained by drawing growth curves showing the increase in dry weight of each organ (e.g. Fig. 8), and reading the weight opposite the middle point of each monthly space. Rates of respiration were corrected to the average monthly temperatures shown in Table 6. Even when this calculation has been made it does not greatly affect the percentage dry weight in the reproductive parts, as compared with the whole plant (see Tables 3 and 6). Respiration intensity of the flower is much greater than that of any other organ, but the total dry weight of the flowers is never very great, usually about 0·5 g.

Table 7 shows the amounts of total carbohydrate, of ash and of total nitrogen in the various organs at significant times during the period of growth. The most important figures are those for total carbohydrate in the rhizome or root, namely, about 60% in September, and about 30% in the following February, a change which can be explained by the manner of growth of the underground parts. Carbohydrate food is stored in a taproot in autumn, but rhizomes grow out from this root during the winter months. By the following February, the system is extensive, but the carbohydrate content has been reduced to 30%. All this growth takes place when the above-ground shoot is dormant. It is significant, however, that the initiation of flower-bud formation on 23 June took place at a time when the amount of food reserves in the plant was at a minimum. It does not appear that any great preponderance of carbohydrate food is necessary to bring about the qualitative change from vegetative to floral determination of the growing-point in *Epilobium angustifolium*.

TABLE 4. *Distribution of dry weight amongst the various organs of* Epilobium angustifolium

	25 Feb. Beginning of shoot growth. Av. of 40 buds	23 June Flower initiation. Av. of 9 shoots	2 Aug. Maximum flowering. Av. of 2 shoots	24 Sept. End of shoot growth. Av. of 5 shoots
	g.	g.	g.	g.
Rhizomes or roots	0·6	2·47	3·05	6·82
Stems	—	0·81	4·35	23·80
Leaves	—	1·03	3·90	14·30
Flowers	—	—	0·50	0·42
Fruits	—	—	—	6·82
Totals	0·6	4·31	11·80	52·16

TABLE 5. *Relative rates of respiration of various organs of* Epilobium angustifolium, 24 *Sept.* 1939. *Temp.* 18° C.

	Fresh weight g.*	Dry weight g.*	% water content*	Mg. of carbon dioxide per g. of fresh weight per hr.	Mg. of carbon dioxide per g. of dry weight per hr.
Roots	110·0	34·1	70·29	0·393	0·132
Stems	415·0	119·0	71·33	0·710	0·208
Leaves	220·0	71·5	67·54	1·92	0·703
Flowers	9·5	2·11	77·80	3·36	1·52
Fruits	102·5	34·29	66·56	2·56	0·799

* The figures in these columns are obtained from estimations of a plant bearing five main stems. Estimations of the relative rates of respiration were made upon 10–20 g. of fresh weight of each of the different organs, and the figures in the last column are calculated upon the percentage water contents shown.

TABLE 6. *Amounts of dry weight of* Epilobium angustifolium *accounted for by respiration of the various organs*

		Roots		Stems		Leaves		Flowers		Fruits	
	Av. temp. ° F.	Av. dry wt. g.	Resp. g.	Av. dry wt. g.	Resp. g.	Av. dry wt. g.	Resp. g.	Av. dry wt. g.	Resp. g.	Av. dry wt. g.	Resp. g.
Mar.	41·6	1·0	0·028	0·25	0·009						
Apr.	45·9	1·4	0·047	0·45	0·024	0·5	0·091	—	—	—	—
May	51·5	1·8	0·080	0·55	0·038	0·7	0·166				
June	56·4	2·4	0·139	0·90	0·076	1·0	0·280	—	—	—	—
July	60·0	2·8	0·173	2·75	0·252	2·5	0·772				
Aug.	60·8	4·0	0·230	10·0	0·918	6·5	2·011	0·5*	0·374	2·0	0·709
Sept. (1–24)	56·7	6·0	0·321	20·0	1·663	12·5	3·504	0·46*	0·345	5·5	1·766
	Totals		1·018		2·980		6·824		0·719		2·475

* 15 July–15 Sept.

	g.
Total amount of dry weight respired during the growing season	14·046
Total amount of dry weight in the plant, 24 Sept. (see Table 4)	52·16
Total amount of dry weight formed by the plant	66·206
Amount of dry weight respired by flowers and fruits	3·224
Amount of dry weight in the flowers and fruits, 24 Sept. (Table 4)	7·24
Total amount of dry weight formed by the flowers and fruits	10·464

% reproductive dry weight of total dry weight = 15·85.

TABLE 7. *Distribution of carbohydrate, nitrogen and ash constituents amongst the various organs of* Epilobium angustifolium

	25 Feb. Beginning of shoot growth			23 June Flower initiation			24 Sept. End of shoot growth		
	Total carbohydrate %	Total nitrogen %	Ash %	Total carbohydrate %	Total nitrogen %	Ash %	Total carbohydrate %	Total nitrogen %	Ash %
Rhizome or root	34·37	3·37	8·71	22·70	2·78	13·20	62·20	1·70	8·79
Stem	—	—	—	22·78	2·70	8·67	26·50	3·82	6·22
Leaves	—	—	—	22·36	5·70	8·51	25·98	4·71	8·49
Fruits and seeds	—	—	—	—	—	—	28·60	2·13	5·42*

* The ash content of flowers was determined on a sample collected 17 Sept. 1938, and found to be 4·65 %.
All figures are given as percentages of dry weight.

THE EFFECTS OF INADEQUATE NUTRITION FOR THE DEVELOPMENT OF FLOWER INITIALS

The production of flower initials pledges a certain amount of future development. Are the nutritional needs of such future development always met by the plant, and if not, what happens? It has been suggested that the general nutrition of the lupin is often inadequate for the ripening of fruits from all flowers of the spike, for there are often considerable lengths of flower stem without fruit. The flowers are cut off by an abscission layer formed at the base of the pedicel, but the shoot often recovers and forms a further series of fruits above the bare stem. Similarly, very floriferous trees of pear, apple, laburnum, sycamore, horse-chestnut and others, often shed a large part, or even the whole, of their blossom, and again the individual flowers are cut off by an abscission layer at the base of the flower stalk (Fig. 7).

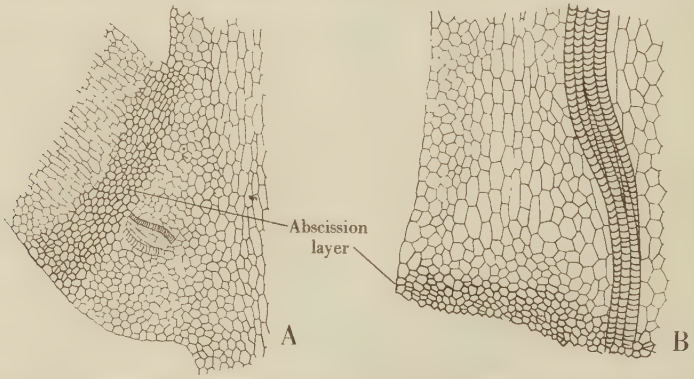

Fig. 7. Formation of an abscission layer across the base of a flower stalk of the pear (A) × 60, and evidence of such a layer upon part of the base of a shed flower stalk (B) × 150.

It was also ascertained, in the pear, by microscopic examination, that the ovaries of such flowers as dropped from the plant had been fertilized. A certain amount of carbohydrate food is stored in the wood of fruit trees during the winter, and this must be used by the developing flowers when they open in spring, since the leaves of most tree fruits are only just beginning to emerge when the flowers are fully open. Fertilization initiates a period of vigorous growth of the ovary, with demands for food which cannot, under the circumstances, be met. If this view is correct, it ought to be possible to retain flowers upon the tree by the

simple expedient of reducing their number at an early stage. Artificial reduction of the numbers should leave more adequate nutrition for the remainder.

This was accordingly tried upon three bush apple trees, variety Bramley's Seedling, growing at Slaithwaite, near Huddersfield, in 1932. These trees had been extremely floriferous for several years, but never set fruit. Half the main branches on each tree had all the flowers except one removed from each truss, as soon as they could be distinguished from each other. The other branches were left untreated, and followed the usual course of cutting off their flower buds, with no setting of fruit. The branches with a reduced number of flowers each set an average of five apples, which increased in size normally, until a high wind in early August unfortunately removed them when a diameter of 2–2½ in. had been attained.

Since normal development can only proceed when the number of developing flowers is reduced, the inference is that the amount of food available was insufficient for the original total number.

The effect of flowering on metabolism

When the rates of growth of *Epilobium angustifolium* are compared with the external factors which are known to affect the vigour of photosynthesis (Fig. 8), it does not seem possible to find any causal relation between the two. The greatest growth takes place when

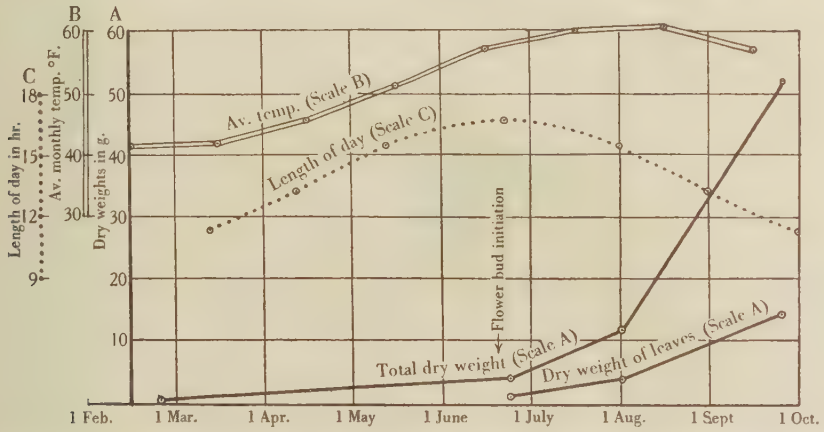

Fig. 8. Increase in dry weight of *Epilobium angustifolium* in relation to average temperature and length of day.

the average temperatures are falling, and when the daily period of sunlight is becoming shorter (Fig. 8). There is, moreover, no disability of delayed nocturnal translocation which might cause increasing growth in shorter days, as in the late chrysanthemum. A sharp increase in the rate of growth can, however, be observed, from the time of first initiation of flower buds in the growing apex. Further light is shed upon this question by experiments which were initially designed to test for the presence of a possible flowering hormone.

Five young vegetative plants of *Sisymbrium alliaria* were inoculated with the extract of a flowering shoot of the same species on 10 June 1939. The extract was prepared by grinding the flowers and flower stalks in a mortar, adding water equal in volume to the ground material, and then filtering the extract through ordinary filter paper. A hypodermic syringe was used to introduce the extract into the bases of the smallest leaves and other tissues immediately surrounding the growing-point. Five pricks were given per plant, but it is impossible to say exactly how much liquid actually reached the tissue, as some was always forced out of the needle prick.

The results of this experiment are shown by Fig. 9. The inoculated plants grew considerably larger than the uninoculated ones. Only four out of the five treated plants, however, appeared to show benefit from inoculation, and it is possible that the results would have been more striking if all the inoculations had been successful. The plants remained entirely vegetative until the usual time of floral determination in January, so no effect of a flower-forming hormone was demonstrated. It appears clear, however, that the extract of a flowering shoot had a potent effect in greatly increasing the rate of growth of a vegetative plant. This is in line with the previous observation on *Epilobium angustifolium*, but it shows that the increase of growth which accompanies the onset of flowering can be brought about by an actual substance which can be separated from the plant.

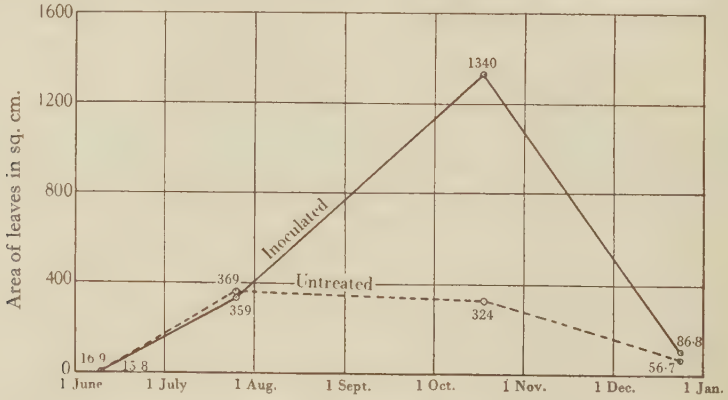

Fig. 9. Relative rates of growth of *Sisymbrium alliaria*, as judged by leaf area.

Discussion

Eleven plants which severally make their flower initials at all times of the year have all mobile carbohydrates. There is no evidence that those species which begin to make their flowers during the short days of autumn and winter do so because of a disability of nocturnal translocation, as was the case with late-flowering chrysanthemum and soy bean (Grainger, 1938). It may be that such delay in nocturnal translocation is characteristic of certain "short-day" cultivated plants of tropical origin. Most of the species used in the present investigation are native British species, or have been introduced to cultivation from temperate regions.

Most of the plants show a marked lowering of total carbohydrate during the hours of darkness, thus demonstrating that considerable amounts are transported from the leaves at night. *Centaurea nigra*, *Salix caprea* and *Saxifraga tridactylites*, however, apparently maintain their content of total carbohydrate with but slight fluctuation. It is the qualitative determinations which supply the evidence of mobility of the carbohydrate in these plants, by revealing the presence of reducing sugars at all time. The carbohydrate would thus, presumably, always be available for transport to the growing-point. Both mechanisms of translocation appear to be equally effective for the initiation of flower formation, for both support direct-flowering and indirect-flowering plants (Grainger, 1939), and both allow the floral determination of the growing-point during autumn or winter, when photosynthesis would be at a minimum.

The evidence given on p. 319 shows that the onset of flowering in *Epilobium angustifolium* is connected with a marked increase in the rate of growth of the whole plant (Table 4 and Fig. 8), but the experiment with *Sisymbrium alliaria* in the same section suggests that it is resultant rather than causal. A certain substance appears to be characteristic of the flowering state, but not of the vegetative phase, in this plant. Such a substance could hardly be the cause of flowering, for in *Sisymbrium alliaria* its inoculation into the vegetative seedling increased only the rate of vegetative growth. Its presence is, however, indicative of a different kind of metabolism to that found in the vegetative state. The effects of inoculation appeared to diminish after several months, showing that the vegetative plant itself had not yet formed the substance. Dearborn (1936) has published detailed work upon a similar question. He found that fruiting plants of the cucumber had a higher rate of vegetative growth for 10–14 days after pollination than had deflorated plants. Amino- and amide-nitrogen accumulated in plants the flowers of which had been removed.

The inoculation of vegetative plants with extract from a flowering spike of the same species offers considerable practical possibilities. If the rates of growth of cabbages, brussels sprouts and plants of like habit could be increased by the treatment, a useful amount of control would be afforded to the gardener: experiments are in progress to test this question further. Another finding of practical significance in this paper is that very floriferous fruit trees which do not set fruit may be caused to yield by thinning the flowers in each truss to one or two, as soon as they can be distinguished from one another.

A plant in general expends only a small percentage of its total food production on flowering (p. 316), though it may thereby lay the foundations for more future development than the plant can meet (p. 318). Floral initiation in *Epilobium angustifolium*, moreover, coincided with the time when reserves of food in the plant were at a minimum (p. 316). It would appear unlikely that any great preponderance of one metabolic product over another could be a major cause of flowering. The picture is rather one of a more delicate and qualitative balance, in which the relative rates of photosynthesis and of growth must also be known.

It was suggested by Grainger (1939) that a plant could not flower in the early months of the year unless it was evergreen or possessed a store of food. This refers to the later development and emergence of the flower, whereas the suggestions in the last paragraph relate to the earliest time in the history of a flower, namely, the time when the growing-point ceases to form leaf initials and begins to organize the flower. Grainger (1939) pointed out that flower initiation and emergence are governed by quite different sets of conditions, and the present results strengthen this conclusion.

Summary

1. Mobile carbohydrate has been demonstrated in eleven species of plants native in temperate regions, and which severally form their flower initials at all times of the year. No delay in nocturnal translocation was found in such species (*Saxifraga tridactylites* and *Sisymbrium alliaria*) as are florally determined in the short days of autumn and winter, as was demonstrated in an earlier paper, for the late-flowering chrysanthemum and soy bean, which exhibit typical "short-day" behaviour.

2. A plant generally expends only a small percentage of its dry weight in the production of flowers.

3. The production of flowers may, however, pledge such a large amount of future development that the plant is unable to meet all metabolic demands. Flowers then fall by abscission at the base of each pedicel. This loss can be prevented in the apple by thinning the flowers to one or two per truss, as soon as they can be distinguished.

4. Evidence of a close relation between the onset of flower formation and a marked increase in the rate of vegetative growth is presented for *Epilobium angustifolium*, and it has been found possible to stimulate the rate of vegetative growth of first-year plants of *Sisymbrium alliaria* by inoculating them with extract from an inflorescence of the same species.

5. Flower initiation in *Epilobium angustifolium* occurred at the time when the amount of stored carbohydrate in the plant was at a minimum.

6. These results strengthen a previous conclusion that flower initiation and flower emergence are governed by quite different sets of conditions. It seems unlikely that flower initiation is caused by any considerable preponderance of one metabolic product over another. There is evidence, however, that a more delicate and qualitative difference in metabolism characterizes the change from vegetative to floral organization of the growing-point.

The writer wishes to acknowledge the valuable help of Mrs M. Grainger in all stages of this investigation, of Prof. J. H. Priestley, of Leeds University, for facilities in his laboratories, of Mr F. Barnett, of Leeds, with the preparation of microscope sections, and of Mr E. W. Aubrook, of the Tolson Memorial Museum, Huddersfield, with some of the meteorological data.

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SOIL CONDITIONS AND THE *FUSARIUM CULMORUM* SEEDLING BLIGHT OF WHEAT

By C. I. SHEN, PH.D.

Department of Plant Pathology, Rothamsted Experimental Station, Harpenden, Herts

ATTENTION was directed to brown foot rot of cereals, caused by *Fusarium* spp., by the unusual prevalence of this disease in the southern and eastern counties in the dry season of 1938. Schaffnit (1930) in Germany and Machacek & Greaney (1935) in Canada have commented upon the association of this trouble with dry years. *Fusarium* seedling blight and foot rot appear to be of most importance in the northern counties of England, where their occurrence is perhaps to be associated with the prevalence of acid soils (Bennet, 1939); Blair (1937) demonstrated a connexion between *Fusarium* seedling blight and soil acidity by means of a field survey in New Zealand. Machacek (1936) reported an association between severe local outbreaks of *Fusarium* foot rot and excess of soluble salts in the soil in Canada. Greaney (1938) was unable to demonstrate any relation between *F. culmorum* seedling blight and deficiency of phosphate in nutrient sand cultures, but found that excess of phosphate tended to increase the severity of the disease. Machacek & Greaney (1936) showed that mechanical injury of the seed greatly predisposed seedlings to the *Fusarium* disease. Earlier experiments on the relation of soil temperature and moisture to the disease have been discussed by Garrett (1934).

The foregoing observations suggest that injury to the cereal plant, whether by drought, soil acidity, excess of soluble salts or mechanical damage to the seed, predisposes the plant to *Fusarium* infection. The search for those predisposing factors has been intensified by Sadasivan's (1939) discovery that *F. culmorum* appears to be a regularly occurring soil inhabitant, especially important in the decomposition of cereal residues, so that the occurrence of the disease can scarcely be correlated with the presence or absence of the parasite. The present paper reports experiments on the effect of soil conditions upon the seedling blight stage, and is a necessary preliminary to any study of the brown foot rot stage of the *F. culmorum* disease.

EXPERIMENTAL

The experiments were carried out in glass tumblers, using Garrett's technique (1938): Harpenden allotment soil and sand, respectively, were used for most of the experiments. Stocks of soil were air-dried, crushed, and passed through a 1·8 mm. mesh sieve; the saturation capacity of each stock, after thorough mixing, was determined by the perforated box method. In setting up an experiment, quantities of soil approximately equivalent to a volume of 180 c.c. were weighed into the tumblers, and the correct amount of water or nutrient solution necessary to bring the soil to the desired degree of saturation was added slowly to the soil in each tumbler from a measuring cylinder. For moisture contents of 50 % saturation, or more, a uniform distribution of soil moisture was thus obtained by some 48 hr. after filling. For moisture contents of less than 40 % saturation, soil and water were mixed by hand in a basin, and the moist soil then weighed into the tumblers. Seven seeds of Little Joss wheat were planted per tumbler; the method of inoculation varied with individual experiments, but after planting, seeds were covered with 40–60 g. of the same soil. By means of this variation in the weight of covering soil, and by the selection of different weight classes of tumblers for different soil

series, it was generally possible to bring all tumblers of an experiment to one of two or three final weights, which facilitated the periodical watering. The tumblers, wrapped in black paper, were randomized on the glasshouse bench, and until emergence of the seedlings were covered with glass lids lined with moist filter paper. Plants were harvested and recorded 2 to 3 weeks after planting.

In estimating the amount of disease, each plant was placed, by inspection of shoot and root system floating in water over a white ground, in one of the following seven infection classes:

Degree of infection	Numerical rating
No infection	0
Small lesions on roots or base of stem	1
Larger lesions on roots or base of stem	2
Plant yellowed and visibly stunted with severe basal lesion	3
Plant wilted and dying	4
Plant killed after emergence	5
Pre-emergence killing of seedling	6

The disease rating for any experimental series was obtained by averaging that for the individual plants comprising it, and expressed as a percentage of the maximum possible rating (6). The percentage of pre-emergence killing in any series was estimated on the basis of 94% emergence in the stock of Little Joss seed employed throughout this work.

The isolate of *Fusarium culmorum* was originally obtained from maize, being kindly supplied to the writer by Dr F. T. Bennett. A spore suspension was always employed as inoculum in order to avoid the microbiological complications due to addition of cornmeal or other organic inocula (Garrett, 1934). The spore suspension was washed off one-month bottle slant cultures on potato-dextrose agar, and the density estimated by haemocytometer count, being subsequently adjusted by dilution if necessary. In some experimental series the seed was steeped in spore suspension before planting; in other series, 2 c.c. of the spore suspension was added to each planting hole.

(1) *Concentration of spore suspension, and mode of inoculation*

A stock spore suspension was made up, having a density of 267,000 spores per c.c., and diluted to $\frac{1}{2}$, $\frac{1}{10}$, $\frac{1}{100}$, and $\frac{1}{1000}$, respectively, of its original strength. Seed was used both dry and previously soaked for 48 hr. at laboratory temperature (*ca.* 16° C.); the seed was steeped in the respective spore suspensions for 20 min., and then planted immediately in sand at 50% saturation. Results are given in Table 1.

TABLE 1. *Degree of seedling blight: concentration of spore suspension (mean of six tumblers)*

	Dry-planted seed				Germinated seed			
Thousand spores per c.c. of spore suspension	166	29·6	4	0·22	267	166	29·6	4
Disease rating	91	84	65	56	42	43	35	39

In the dry-seed series, intensity of infection is correlated with density of the spore suspension; in the pre-germinated seed series, this relation is scarcely significant, and intensity of infection is much less than that in the dry-seed series.

In a further experiment, dry seed was compared with seed pre-germinated for 42 and 66 hr. respectively, before planting in sand at 50% saturation; inoculation was made by addition of 2 c.c. of spore suspension to each hole before planting. Results are given in Table 2.

TABLE 2. *Degree of seedling blight: dry and germinated seed (mean of six tumblers)*

Treatment of seed	Planted dry	Germinated for 42 hr. before planting	Germinated for 66 hr. before planting
Disease rating	80	49	47

The reduction in intensity of infection effected by pre-germination of the seed is once again apparent, but increase in the period of pre-soaking from 42 to 66 hr. has not reduced infection any further.

An experiment was next set up to test the effect of incubating the inoculated seed under humid conditions and at a temperature of 25° C., for varying periods before planting. A spore suspension of density 23,000 per c.c. was divided into two parts, to one of which 2% glucose was added. Inoculation was effected by evacuating the dry seed in the spore suspension. Incubation periods of 0, 10, 20 and 30 hr. at 25° C. were employed; half the seed of each batch was planted immediately, and the other half air-dried and planted 2 weeks later. Sand at 50% saturation was employed as the planting medium throughout. Results are given in Table 3.

TABLE 3. *Degree of seedling blight: period of incubation (mean of six tumblers)*

	Spore suspension in water				Spore suspension in 2% glucose			
Incubation of inoculated seed	None	10 hr.	20 hr.	30 hr.	None	10 hr.	20 hr.	30 hr.
Disease rating at 1st planting	92	92	100	100	100	100	100	100
Disease rating at 2nd planting	76	59	71	87	72	36	47	82

When the seed was planted straight away, infection was very severe; some 90–100% of seedlings were killed in every treatment. The effect of an incubation period before planting showed only in the spore suspension without glucose. When the seed was dried after the various incubation periods, and planted 2 weeks later, infection was less severe, and interesting differences were found. The intensity of infection was considerably decreased by an incubation period of 10 hr., somewhat decreased by the 20 hr. incubation, and increased only by the 30 hr. incubation period. It seems likely that the short incubation period of 10 hr. was just sufficient for germination of perhaps a majority of the spores; these might then have proved less tolerant of the sudden drying than the non-germinated spores of the no-incubation series. The 30-hr. incubation period, on the other hand, would have been sufficient for the establishment of a young mycelium, which would probably have withstood the effect of sudden drying as well as the non-germinated spores.

(2) *The limitation of seminal root infection*

An experiment was set up with the object of estimating the extension of infection by *F. culmorum* along the seminal roots under different soil conditions. Sterile sand, unsterilized sand and unsterilized allotment soil, all ± 1% glucose and adjusted to a moisture content of 50% saturation, were employed in this experiment. Inoculation was made by addition of 2 c.c. of a spore suspension containing 1500 spores per c.c. to each hole before planting. The degree of infection in these different series is given in Table 4.

TABLE 4. *Degree of seedling blight: length of root infected (mean of six tumblers)*

Soil type and treatment	Sterilized sand	Sterilized sand + glucose	Unsterilized sand	Unsterilized sand + glucose	Unsterilized allotment soil	Unsterilized allotment soil + glucose
Disease rating	44	65	34	23	10	6
Mean length in mm. of root infected by *F. culmorum*	10·2	32·9	2·9	7·3	1·7	0·5
No. of roots measured	149	168	136	156	164	176

Intensity of infection is greatest in the sterile sand, next in the unsterilized sand, and least in the unsterilized soil. The effect of glucose in increasing infection under sterile conditions, but decreasing infection in the unsterilized sand and soil, is by now a well-known "antibiosis" effect. Measurements were also made of the mean length of seminal root infected, from the seed downwards, in the different series. The extent of visible discoloration was measured under the binocular dissecting microscope; previous microscopical examination of cut sections had shown that the limit of infection coincided fairly closely with that of visible discoloration. These data are also given in Table 4.

It seems evident from these figures that *F. culmorum* has little power of spreading along the plant roots in the manner either of the vascular wilt *Fusaria* or of *Ophiobolus graminis*. Where extension of infection has occurred over an appreciable length of root, it can be put down to growth of the organism through the soil. This apparent inability of *Fusarium culmorum* to spread directly along the seminal roots, whether inside or outside, is of great interest, and helps to explain the limitations of its parasitism; it has been observed that if a germinating wheat seedling survives the first intensity of attack by the fungus, it frequently recovers completely, and develops into a healthy plant.

(3) *Soil moisture content and infection*

An experiment was set up with allotment soil and sand, respectively, adjusted to three different moisture contents, viz. 30, 50 and 80% of saturation. Dry seed was inoculated by steeping in a spore suspension of density 2500 spores per c.c. Results are given in Table 5.

TABLE 5. *Degree of seedling blight: moisture content (mean of three tumblers)*

	In allotment soil			In sand		
Moisture content as % saturation	30	50	80	30	50	80
Disease rating	100	37	28	91	66	55

Infection was most severe at the lowest moisture content in soil, and decreased with rise in moisture content. The severe infection at low moisture content must be attributed in part to the adverse effect of low moisture upon the wheat seedling, but it must be intensified by the improved aeration conditions, which favour the fungus. It may be for this reason that rising soil moisture reduces infection to a greater extent in soil than in sand, which remains well aerated even at the higher moisture contents.

(4) *Soil reaction and infection*

A first experiment was carried out in sand culture at a moisture content of 50% saturation. The nutrient solution of Gregory & Crowther (1928) was employed, different *p*H values being secured by the addition of sodium hydroxide and sulphuric acid, respectively. An inoculum of 2 c.c. of spore suspension of density 22,000 per c.c. was poured into each planting hole. The results are given in Table 6.

TABLE 6. *Degree of seedling blight: soil reaction (mean of six tumblers)*

Initial *p*H of sand	3·7	4·4	5·8	6·6	6·9	7·3	8·1
Final *p*H	3·9	5·7	6·1	6·1	7·3	7·4	7·8
Disease rating	81	93	93	64	59	48	87

Intensity of infection was lowest at *p*H 7·3, and increased with acidity of the sand culture to *p*H 4·4; with a further increase of acidity to *p*H 3·7, intensity of infection declined somewhat. On the other hand, severity of infection also increased markedly with rise in *p*H value to 8·1.

In a second experiment, different reactions were obtained by addition of sodium hydroxide and sulphuric acid, respectively, to allotment soil, which was then incubated at a moisture content of 50% saturation in loosely covered flower pots in the glasshouse for 2 weeks. The treated soils were then dried, crushed and sieved, in preparation for use in the tumblers. The experiment was conducted at the usual soil mosture content of 50% saturation; inoculation was effected by steeping the dry seed in a spore suspension of density 12,500 per c.c. Results are given in Table 7.

TABLE 7. *Degree of seedling blight: soil reaction (mean of three tumblers)*

	+1·8% H_2SO_4	+1·3% H_2SO_4	+0·8% H_2SO_4	No treatment	+0·3% NaOH	+0·6% NaOH	+1·2% NaOH
Initial *p*H	4·4	5·7	6·4	7·1	7·3	7·9	8·1
Final *p*H	4·9	5·9	6·2	6·7	7·1	7·8	8·1
Disease rating	55	47	23	42	34	43	74

Intensity of infection in this experiment was lowest at *p*H 6·4 and increased on the one hand with acidity to *p*H 4·4, and on the other with alkalinity to *p*H 8·1.

It seemed possible, in view of the absence of any reported association between *F. culmorum* seedling blight and alkaline soils in the field, that the sodium hydroxide might be exerting a directly injurious effect upon the wheat seedling, and hence predisposing it to infection. In a third experiment, therefore, calcium hydroxide was compared with sodium hydroxide. The air-dry untreated allotment soil was weighed directly into the tumblers, and the requisite amounts of sodium hydroxide and sulphuric acid, respectively, added in solution. An additional series of tumblers was filled with soil previously mixed dry with finely divided calcium hydroxide. Soil moisture content was adjusted to 60% saturation, and all tumblers incubated in the laboratory for 1 week before planting. The dry seed was inoculated by steeping in a spore suspension of density 9000 per c.c. Results are given in Table 8.

TABLE 8. *Degree of seedling blight: soil reaction (mean of six tumblers)*

	+0·8% H_2SO_4	+0·6% H_2SO_4	+0·3% H_2SO_4	No treatment	+0·5% NaOH	+0·5% $Ca(OH)_2$
Initial *p*H	3·7	3·9	5·0	6·9	8·4	8·5
Disease rating	55	42	31	23	91	23

Whereas infection was higher in the sodium hydroxide series than in any other, infection in the calcium hydroxide series was no higher than that in the untreated soil. The growth of the control plants in the sodium hydroxide series in this experiment was very poor indeed; 18% of the plants died, and the non-inoculated controls received a disease rating of 67, as compared with a rating of 4 for the control plants both in the untreated and in the limed soil. In the preceding experiment, conditions would have permitted greater reaction between the sodium hydroxide and the soil before the start of the experiment, and hence a smaller injurious effect on the seedlings.

The results of these three experiments therefore support the field observations of Blair (1937) as to the prevalence of *Fusarium* seedling blight on the more acid soils in New Zealand.

(5) *Plant nutrition and infection*

The effect of plant nutrition upon infection was investigated in sand culture. Acid-treated and washed sand was brought to a moisture content of 50% saturation by addition of the nutrient solution of Gregory & Crowther (1928), which was used, however, at double strength, viz.:

$NaNO_3$	3·04 g./l.	$MgSO_4.7H_2O$	0·42 g./l.
$Na_2HPo_4.12H_2O$	1·0 ,,	Boric acid	0·002 ,,
K_2SO_4	0·62 ,,	$MnSO_4$	0·002 ,,
$CaCl_2$	0·16 ,,	Fe-tartrate	0·28 ,,

The manurial treatments comprised full nutrient solution, no nutrients, and elements nitrogen, phosphorus, and potassium singly and in pairs, giving presence and absence of N, P, and K. For inoculation, 2 c.c. of a spore suspension, of density 18,000 per c.c., was added to each planting hole. In order the better to demonstrate any effect of the nutrients upon seedling resistance, pre-germinated seed was planted, to reduce killing of the seedlings to a minimum (see Tables 1 and 2). The degree of replication was increased from the usual six to ten tumblers per series in this experiment, making seventy plants for each treatment. Results are given in Table 9.

TABLE 9. *Degree of seedling blight: differential nutrition* (*mean of ten tumblers*)

	Nil	N	P	K	NP	NK	PK	NPK
% pre-emergence killing	1	0	3	1	6	3	0	6
% post-emergence killing	29	0	4	23	3	1	17	0
Disease rating, excluding pre-emergence killing	57	33	41	55	34	37	46	33

Intensity of infection is highest in the no-nutrient series, and lowest in that receiving full nutrients. Addition of N, either alone or in combination with P or K, seems to be almost or quite as effective in reducing infection as the full nutrient solution. P, either alone or in combination with K, is markedly less effective in reducing infection than is N. K alone has not significantly reduced infection below that in the no-nutrient series; there is a suggestion that in combination with N and P, respectively, it actually increases infection above that occurring with either N or P alone.

SUMMARY

The influence of soil conditions upon the *Fusarium culmorum* seedling blight of wheat has been studied by means of the glass tumbler technique. Intensity of infection increased with density of the spore suspension used to inoculate the seed, but was reduced by germinating the seed for 48 hr. or more before inoculation. Infection was most severe at a low soil moisture content and in acid soil. In nutrient sand culture, infection was highest in the no-nutrient series and lowest in that receiving full nutrients. Nitrogen alone appeared to be as effective in reducing infection as the full nutrient solution, phosphorus was less effective, whilst potassium had no beneficial effect at all upon seedling resistance.

This work was commenced in the Department of Plant Pathology, Imperial College of Science and Technology, and continued at the Rothamsted Experimental Station. The writer is indebted to Prof. W. Brown and Mr S. D. Garrett, respectively, for suggesting the problem, and for advice and direction during the progress of the investigation.

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OBSERVATIONS ON THE DISEASE OF SEA LYME-GRASS (*ELYMUS ARENARIUS* L.) CAUSED BY *USTILAGO HYPODYTES* (SCHLECHT.) FRIES.

By T. E. T. BOND, PH.D.[1]

Department of Botany, Aberdeen University

(With Plates 12 and 13 and 3 Text-figures)

USTILAGO HYPODYTES was recorded by Berkeley & Broome (1841, 1850) and occurs on *Elymus arenarius* and *Agropyrum* species. A knowledge of its life-history on these plants appeared desirable in view of the possibility of an extension in host range to nearly related cereals, added emphasis being given to this viewpoint by the recent production of perennial *Triticum-Agropyrum* hybrids. *Elymus arenarius* is widely planted to prevent coastal erosion, and no knowledge was available of the effect of the fungus on its suitability for this purpose. The virtual absence of seed production by infected plants is known. In America, *Ustilago hypodytes* was described by Fischer (1938) as an increasing menace to the cultivation of *Agropyrum cristatum* (L.) Beauv. and other valuable forage grasses. The disease on *Elymus* was studied by Bornhövd (1936), who gave a full summary of the literature. She described the process of spore germination and the growth and nuclear behaviour of the fungus on artificial media, but dealt less adequately with the symptoms and spread of the disease and the histology of infection: her inoculation experiments were inconclusive.

SYMPTOMS AND EFFECTS

External morphology of diseased shoots

Wilson (1910) states "...in the diseased shoot, in place of the ear, the upper part of the stem, where the ear ought to be, is changed into a series of short nodes, covered for the most part by leaf sheaths without blades. The nodes become shorter and shorter as they ascend, and at the top there is a flattened, deformed structure composed of a number of scales which are placed in a manner recalling to some degree the arrangement of the glumes and paleae of the normal spikelets." Bornhövd (1936), and most other writers with the exception of Magnus (1896), overlooked the homology between the diseased shoots and the normal inflorescences, which is apparent from this account, and regarded the former merely as sterile leafy shoots ("Blattschosse"). Comparisons have been made of the average height and number of internodes of the two kinds of shoot and different results have been obtained by different workers. Bornhövd (1936) tends to regard these discrepancies as indications of the existence of biological specialization within the *Ustilago hypodytes* group.

In the neighbourhood of Aberdeen the healthy flowering shoots were fairly uniform in height in different localities, the average height from the last rooting node being 100 cm. (74–117 cm.). The diseased shoots were more variable (40–166 cm.); in some localities they were considerably shorter than the healthy, in others, taller. Comparisons of the number of internodes of the diseased and healthy shoots are permissible only between corresponding

[1] Now at the Tea Research Institute of Ceylon, St Coombs, Talawakelle, Ceylon.

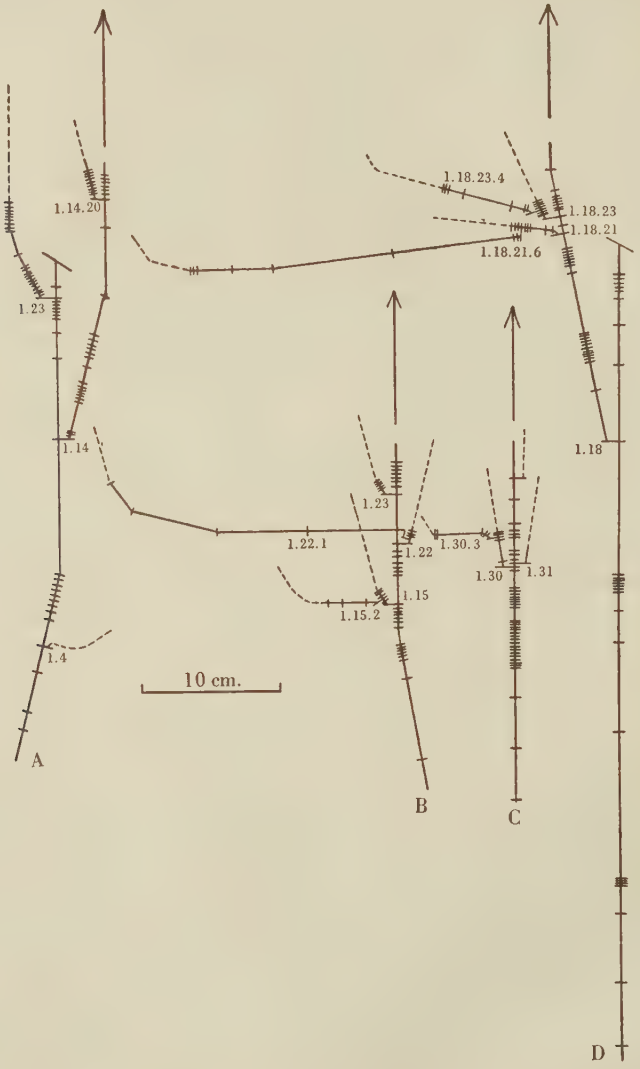
regions of the stem. The flowering stems have from four to six vegetative internodes exceeding 0·5 cm. in length and an average of twenty-three internodes in the inflorescence. The ears average about 22 cm. in length, which is about one-third the length of the vegetative region. In the typical diseased stems which remain sterile, the two regions can be distinguished by their appendages. The vegetative region bears normal leaves; the number of internodes is about the same but the total length is only one-half that of the corresponding region of the healthy stems. The inflorescence region bears appendages resembling leaf sheaths without blades, which are probably best described as bracts. This region comprises fewer internodes than the normal ear but is much elongated, being about twice the length of the vegetative region. It is here that the fungus chiefly sporulates: its behaviour is thus essentially that of an "inflorescence" smut, which modifies the inflorescence and causes its excessive elongation, apparently at the expense of the vegetative region (Pl. 12, fig. 1). In the lower part of the diseased inflorescence region, the bracts frequently show a greater resemblance to the foliage leaves. Transitional forms occur with either one or two auricles associated with linear extensions of tissue reproducing the structure of the normal lamina. Occasionally the last foliage leaf of the vegetative region is characterized by an abnormally short and narrow lamina. In the uppermost internodes, the bracts are associated with minute axillary structures resembling abortive spikelets, which are usually covered with spores. The diseased stems may become partially fertile at their apex (Pl. 12, figs. 2, 3). Here, the axillary structures bear an increasing resemblance to normal spikelets and on them sporulation is reduced. In one plant, spikelets were found in the condition of anthesis, with the fungus sporulating at the base of the paleae, on the filaments of the stamens, and on the ovary (Pl. 12, figs. 4, 5). The bracts become shorter and may disappear entirely. The abnormal flowering axis usually remains more slender than the healthy ear, and may be branched. In the sterile inflorescences, and particularly in the transitional stages to the healthy state, the bracts invite comparison with the short sheath-like outgrowths frequently developed from the lowest one or two nodes of the normal ear and repeated again in a reduced form at each successive node of the rachis. These structures, however, are non-vascular. Similar outgrowths occurring in *Hordeum* and other genera were described by Arber (1934) as the basal or "collar leaf" and "rachilla-flap" respectively. Arber also discussed the significance of occasional ligule-less foliage leaves without distinction between sheath and limb, with which the bracts might be compared.

Near Aberdeen the fungus occurs also on a maritime form of *Agropyrum repens* Beauv. identified as *A. acutum* R. & S., on which a similar range of symptoms is developed (Pl. 12, figs. 6, 7). On the other hand, the disease was not found on *A. junceum* Beauv. nor on *Ammophila arenaria* Link, although both these hosts are plentiful. Its absence on *Ammophila* (noted also by Bornhövd, 1936), tends to support Plowright's (1892) original suggestion that a distinct biologic form infects this host.

Effect on seasonal development

A feature of the disease not previously recorded is its effect on the response of the plant to seasonal changes. The mode of elongation of the rhizomes is affected, as well as the general balance between the vegetative and reproductive phases. These changes were followed by observation of the growing plants throughout the year and by the excavation of the rhizome systems in October and April. Plants excavated were stripped of their dead leaf sheaths and

the growth of the rhizomes was analysed and mapped, node by node. The nodes were numbered successively and lateral shoots or runners were given numbers corresponding to

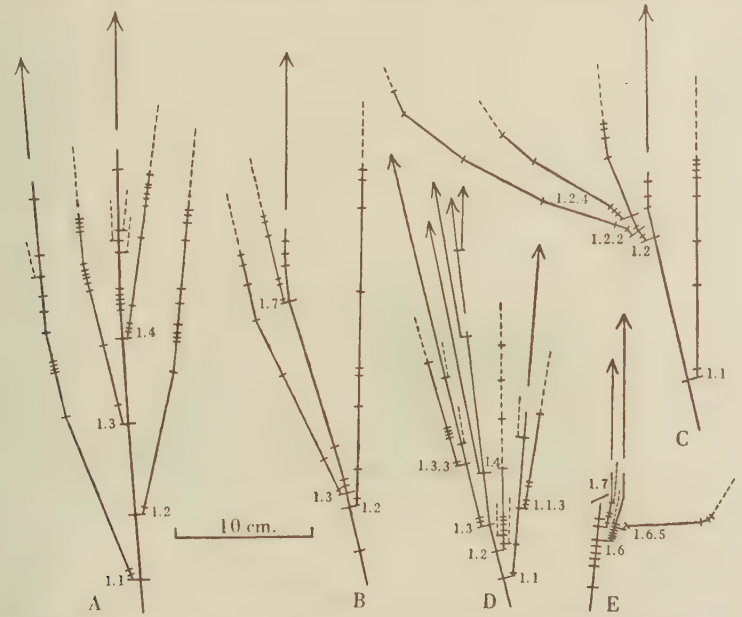


Text-fig. 1. Maps of shoot systems of healthy plants. The short cross lines represent nodes; the long arrows flowering stems, which are interrupted at ground level (i.e. above the last rooting node). Dotted lines represent vegetative apices still enclosed in the leaf sheaths. Flowering stems of a previous year are shown broken off short just above ground level. Shoots are numbered according to the position of their node of origin on the main stem "1". See Pl. 13, fig. 1.

the position of their node of origin. The nodes, lateral buds, and stem apices were preserved in formalin-acetic-alcohol for examination for the occurrence of mycelium. Photographs and maps of some plants excavated are given in Pl. 13, figs. 1–3, and in Text-figs. 1 and 2.

The healthy plants behave in a comparatively uniform manner. Their flowering period is limited to two or three months in the year, the majority of the ears coming into anthesis in the second half of June, remaining until early July, while a small proportion flowers at the beginning of August. The rhizomes have a characteristic habit of growth, showing an alternation of elongated internodes, up to five in number and varying in length from less than 1–10 cm. or more, with groups of numerous short internodes, usually 0·2–0·3 cm. in length (Pl. 13, figs. 1, 2, and Text-fig. 1).

The flowering stems were traced down to their origin with difficulty. Two are represented by shoot "1.14" in Text-fig. 1 A and shoot "1.18" in Text-fig. 1 D. Including the current season's elongation to produce the ear (represented by the arrow), each consists of four elongation periods, and is regarded as the product of four years of active growth. The origin



Text-fig. 2. Maps of shoot systems of diseased plants, constructed as in Text-fig. 1. The long arrows represent spore-bearing stems, which in A, B, C and E are interrupted at ground level, and in D at two nodes above ground level. See Pl. 13, fig. 3.

is intra-vaginal. Additional evidence was obtained from dissection of the apices of leafy stems (in April) about to flower in the current season, as well as from the excavation of former flowering stems which had broken off at ground level (e.g. the main stem "1" in Text-fig. 1 A, D). On the whole, it can be stated that flowering may occur after three years of growth by the shoot, but is commonly delayed until four or five. Lateral shoots and buds may remain quiescent for an unknown period before elongation commences, forming a large number of short internodes at their base (e.g. shoot "1.23" in Text-fig. 1 A), or they may elongate directly (shoot "1.18" in Text-fig. 1 D, as well as the extra-vaginal runners). Extended periods of quiescence may also intervene between successive years in which elongation occurs. Text-fig. 1 C represents a plant in which this may have occurred.

The infected plants produce their spore-bearing stems mostly in June. They appear first rather earlier than the healthy inflorescence but, unlike the latter, continue to be formed in decreasing abundance up to December. Probably, their development is arrested only by the onset of severe frost. This extended period of activity results from a more continuous process of growth in the rhizomes (Pl. 13, fig. 3, Text-fig. 2). The rhythmic development characteristic of the healthy plants is largely obscured. Where it still persists, the nodes marking the periods of quiescence are fewer and less closely crowded (Text-fig. 2A); in other cases, long and short internodes are associated almost at random. The period of vegetative growth normally preceding the elongation of the intra-vaginal shoots to form diseased stems (i.e. the equivalent of inflorescences) tends to be reduced (Text-fig. 2D, E). This leads to a condition of proliferation, where a number of weak, shortened diseased stems appear to rise from a common origin, not far below ground level (Pl. 13, fig. 3). In the extreme case, the buds towards the apex of the leafy part of the stem, which normally remain abortive, develop simultaneously with the parent stem to form short, spore-bearing branches (Text-fig. 2D). In the rhizomes, as in the inflorescence region of the aerial stem, the presence of the fungus favours vegetative elongation at a time when this activity would otherwise be reduced.

Text-fig. 3. Camera-lucida outline of longitudinal section of node with lateral bud at base of diseased stem. Each "×" marks the position of a single hypha or group of hyphae as seen with the $\frac{1}{6}$ in. objective. (The section was 20 μ thick.) Vascular tissue is shaded.

Histology

Certain details in Bornhövd's account may be amplified. Mycelium was detected in every node and associated resting bud of the diseased rhizome systems excavated, as well as in the apices of all shoots and runners. Text-fig. 3 indicates the distribution of mycelium in a single node at the base of a diseased stem (i.e. below the region of sporulation). The concentration of mycelium in the bud is characteristic of diseased plants: hyphae are abundant throughout the developing stem apex and at the base of the protective scale leaves. In the mature stem, they occur chiefly in the outer cortical region, appearing also in the parenchymatous tissue of the nodes and at the base of the leaf sheaths. The hyphae are chiefly intracellular, often somewhat swollen and irregular, and 2–3 μ in diameter (Pl. 13, figs. 4–6). They appear more richly branched and of a greater average diameter in the buds than elsewhere, and the walls appear rather thick in relation to the width of the hyphae. Cross walls are distinguishable (cf. Bornhövd, 1936) and the cells appear uninucleate although outgrowths are occasionally visible which resemble clamp connexions and into which the nuclei appear to be migrating (Pl. 13, figs. 4, 6). In regions of sporulation, the hyphae emerge from the epidermal

walls without constriction, as Bornhövd (1936) observes. Excessive branching with reduction in diameter of the hyphae occurs after emergence and in this way the dense mass of spore-bearing hyphae forming a layer over the surface is built up. Basipetal spore formation is readily observed (Pl. 13, fig. 5) and in favourable sections the origin of the spores inside the hyphae can be detected. Gelatinization of the walls of the hyphae was not apparent, nor were the nuclei distinguished at this stage. Sporulation may occur on the spikelets formed at the apex of the inflorescence region, as well as at the base of the bracts, on the abaxial surface. Here, the spores may be arranged in stripes recalling those formed on its proper hosts by *Ustilago longissima* (Schlecht.) Meyen to which, as Liro (1924) points out, *U. hypodytes* is closely related. However, the spores are still superficial (Pl. 13, fig. 7), as they are on the stem, whereas those of *U. longissima* are erumpent through the epidermis.

Possible modes of transmission

In nature the disease is commonly spread by the vegetative multiplication of affected plants. No shoots or buds from these were found to be free from mycelium either in autumn or spring, and isolated diseased shoots or portions of rhizome detached by wind or currents readily take root and serve as new centres of the disease. Other means of dispersal are bound up with the conditions affecting seed germination and the germination of the spores of the fungus. Seed is ripened locally in limited quantities and can be found on the ripe ears from October to December. Germination outside and in the laboratory was slow and required a minimum time of 3–4 weeks; it rarely exceeded 40%, it was not affected by pre-treatment at 30° C., and was apparently not influenced by burial at various depths. Naturally occurring seedlings were not observed, nor was their occurrence recorded by Trail (1904). Graham (1938), however, states that at St Andrews "in October of each year a green fringe of seedling *Elymus* is found spreading towards the sea". The difference may be accounted for in part by differences in the conformation and stability of the sand dunes. All attempts to germinate the spores of the fungus in damp air or on liquid or solid media gave negative results; both fresh and overwintered spores were used. Seed inoculation experiments, in which seed was dusted with dry spores or evacuated in a spore suspension prior to sowing, or in which clean seed was sown in sand previously mixed with spores or watered with a spore suspension, also failed to give any evidence of germination. A similar failure to obtain germination of the spores of *Ustilago hypodytes* was reported by Plowright (1889); Bornhövd (1936), on the other hand obtained ready germination on a variety of media. The age of her spore material was not stated. Differences in experimental technique or in environmental conditions, or the possible existence of distinct strains of the fungus may be involved. Although experimental evidence on the dispersal of the disease other than by vegetative means has not been obtained, a certain amount of indirect or circumstantial evidence is available.

Systemic infection of the grain. This possibility is raised by the occasional production of normal spikelets by the diseased stems (Pl. 12, fig. 2). In such stems, mycelium was traced for at least three internodes of the flowering rachis beyond the region visibly affected by the disease. Unfortunately, ripe grain was not obtained in two autumns, but immature stages were available in which the organization of the embryo and endosperm was nearly complete. Mycelium was found at the base of the ovary wall, from the time of fertilization onwards, but it never occurred in the ovule or its integuments and in the nearly mature grain it was

confined to the base of the pericarp. There was no indication that the ripening of the grain would be prevented, or its viability impaired. While the possibility of its contamination by dormant mycelium in the grain coat or paleae is not excluded, there seems little likelihood of true systemic infection in the seed itself.

Blossom infection. At the time of flowering, the spores on the diseased stems, although partly exposed (Pl. 12, fig. 1), are firmly compacted and their dispersal at this time seems unlikely. Occasionally, the remains of a few stems produced at the end of the previous year are still in evidence and a small quantity of spores in a dry, powdery condition might be available from these. Ovaries of different ages from healthy plants growing in the vicinity of heavily infected colonies were examined for the presence of spores or mycelium, but none was detected.

Seedling infection. By the time the seeds are ripe, the spores produced during the flowering period are more fully exposed and the masses are drier and fairly powdery. Dispersal of the spores at this stage is proved by their occurrence in quantity as a superficial contamination of the grain (paleae) and empty glumes. Such contaminated grain might be expected to serve as a means of dispersal of the disease, but so far, it has germinated without any evidence for the simultaneous germination of the spores.

Other possibilities. Spores were detected in samples of sand taken at various times of the year. The abundance of spore production, and their gradual dispersal, would result in heavy contamination in localities where the disease is prevalent. Infection of seedlings or of young underground shoots from this source is also possible. The fact that mycelium was on no occasion recorded from individual shoots or runners associated with otherwise healthy plants tends to suggest that infection takes place at the seedling stage rather than later.

Summary

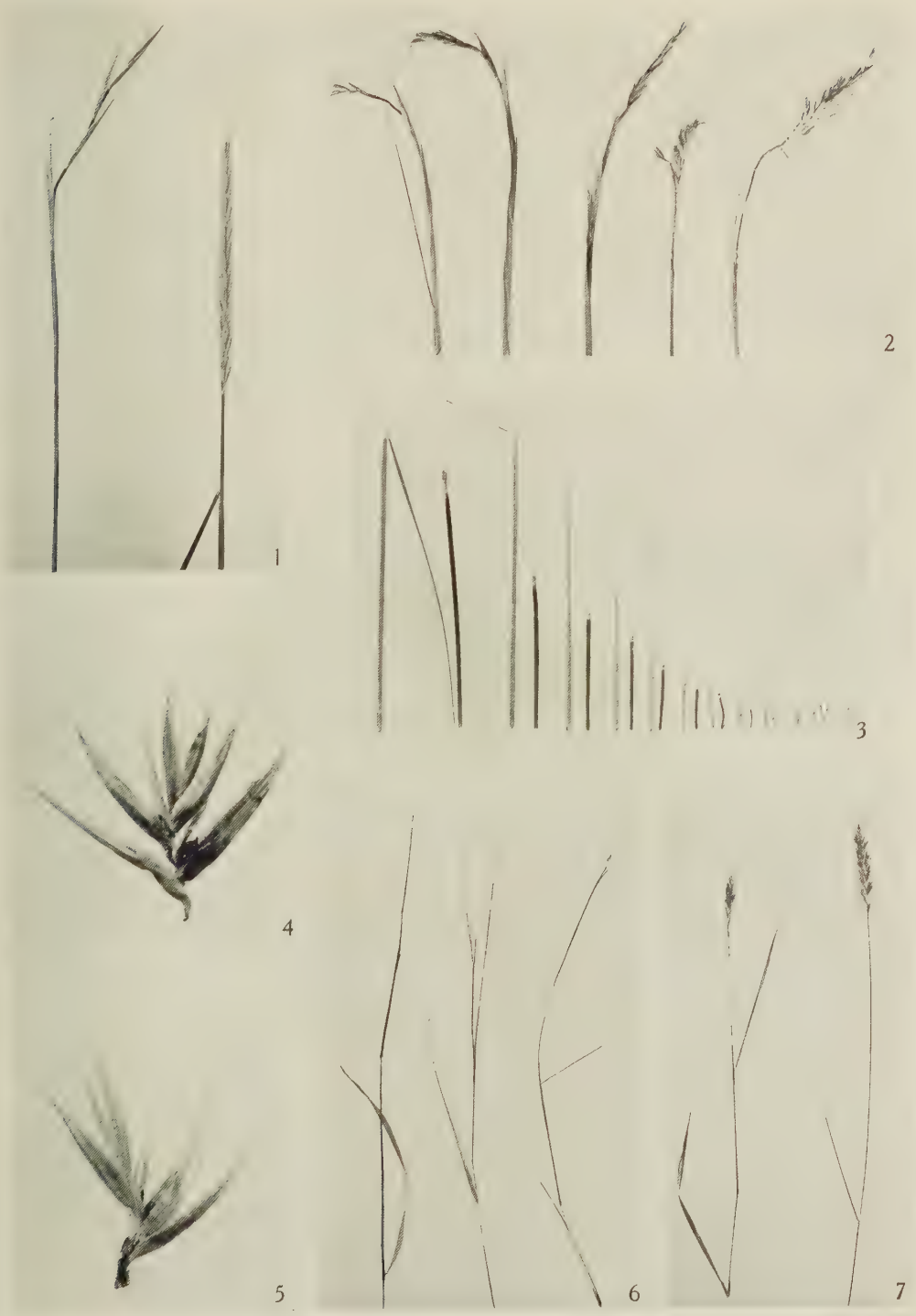
Plants of *Elymus arenarius* L. infected by *Ustilago hypodytes* (Schlecht.) Fries normally remain sterile, producing in place of inflorescences leafy shoots, the morphology of which is described. Occasional diseased shoots produce normal flowering spikelets in their upper part, which may be capable of forming grain. The disease also results in a modification of the habit of growth of the rhizomes, the marked periodicity of growth characteristic of the healthy rhizomes being replaced by a more continuous development. The lateral buds show a tendency to develop more rapidly to form the aerial diseased stems which are the equivalent of inflorescences.

The disease was observed causing similar symptoms on *Agropyrum acutum*, but did not occur on *A. junceum* Beauv. nor on *Ammophila arenaria* Link. in the neighbourhood of Aberdeen.

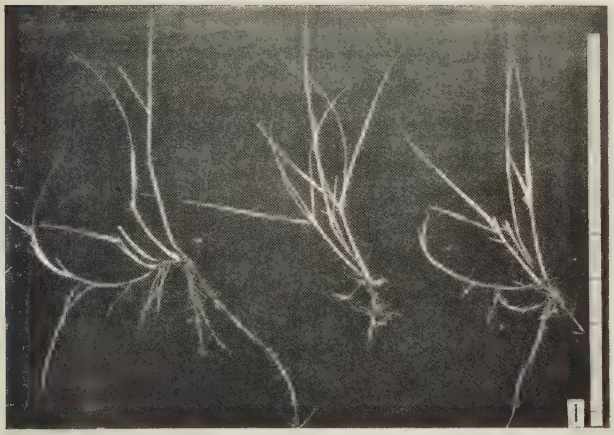
Mycelium was detected in each node of the rhizome and in every lateral bud and stem apex of excavated diseased plants of *Elymus arenarius*. No mycelium occurred in the healthy plants.

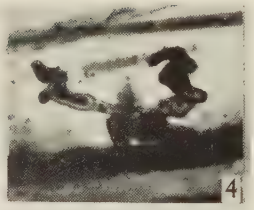
Attempts to germinate the spores proved unsuccessful, and inoculation experiments were inconclusive. Various possible means of transmission of the disease are discussed in the light of the evidence available.

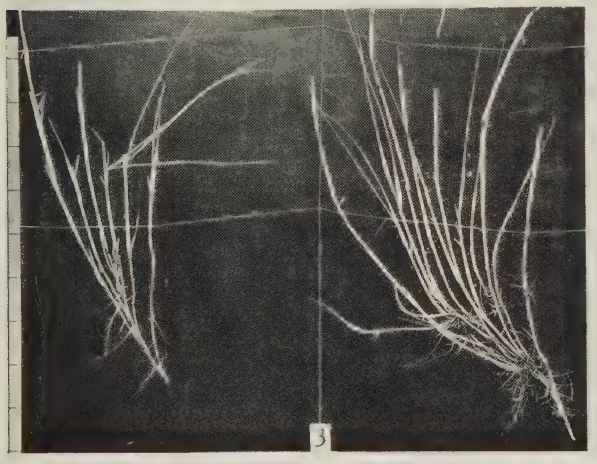
The writer is indebted to Miss K. Sampson for her interest in this investigation and for the various suggestions which she made at the outset, and to Mr I. Moir for assistance with the photographs.

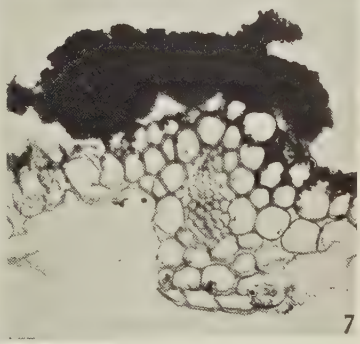


BOND.—Observations on the Disease of Sea Lyme-Grass (*Elymus arenarius* L.) caused by *Ustilago hypodytes* (Schlecht.) Fries (pp. 330–337)









BOND.—Observations on the Disease of Sea Lyme-Grass (*Elymus arenarius* L.) caused by *Ustilago hypodytes* (Schlecht.) Fries (pp. 330–337)

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EXPLANATION OF PLATES 12 AND 13

Plate 12

Fig. 1. Sterile diseased shoot and healthy inflorescence of *Elymus arenarius* L. at the beginning of July. $\times \frac{1}{5}$.

Fig. 2. Diseased shoots, from left to right, showing stages in the development of partial fertility. $\times \frac{1}{4}$.

Fig. 3. Dissection of left-hand shoot in Fig. 2. The leafy appendages are shown with their corresponding internodes. $\times \frac{1}{4}$.

Fig. 4. Diseased spikelet from the second shoot on left in Fig. 2. The lower empty glume has been removed. Note sporulation on the abortive ovary. $\times 1\frac{2}{3}$.

Fig. 5. Another spikelet, as Fig. 4, showing sporulation on the filament of a stamen. $\times 1\frac{2}{3}$.

Fig. 6. Sterile diseased shoots of *Agropyrum caninum.* $\times 7$.

Fig. 7. Partially fertile diseased shoot and healthy inflorescence of *Agropyrum caninum.* $\times 7$.

Plate 13

Fig. 1. Healthy plants, excavated in October after flowering. The plant on the left is analysed in Text-fig. 1 D. $\times 1\frac{1}{4}$ (decimetre scale shown).

Fig. 2. Healthy plant, excavated in April of year after flowering. $\times \frac{1}{10}$.

Fig. 3. Diseased plants, excavated in October. The plant on the left is analysed in Text-fig. 2 A; part of the plant on the right in Text-fig. 2 C. $\times .1\frac{1}{4}$

Figs. 4–5. Intracellular mycelium in stem, showing cross-walls and nuclei. Sections 12μ, stained in Haidenhain's iron-alum haematoxylin and orange G. $\times 330$.

Fig. 6. Intracellular mycelium, and spore formation. As above. $\times 190$.

Fig. 7. Sporulation on bract of diseased inflorescence, transverse section. $\times 59$.

Fig. 8. Details of basipetal spore formation. $\times 330$.

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THE CONTROL OF NARCISSUS LEAF DISEASES
I. WHITE MOULD AND FIRE ON "GOLDEN SPUR"

By P. H. GREGORY[1]

Seale-Hayne Agricultural College, Newton Abbot

As described by Moore (1939) the principal fungus diseases of narcissus foliage known in this country are white mould (*Ramularia vallisumbrosae* Cav.), leaf scorch (*Stagonospora Curtisii* (Berk.) Sacc., smoulder (*Botrytis narcissicola* Kleb.), and fire (*Sclerotinia polyblastis* Gregory). These diseases have been recorded in most areas where narcissus is grown out of doors on a commercial scale, and they are liable to develop with great intensity in districts of high spring rainfall and humidity such as the Isles of Scilly, Cornwall and Devon (Beaumont, 1935). Their occurrence in the early outdoor flower districts of south-west England prompted the investigation to be recorded in these papers. The diseases, their control, and the loss of crop following attack have been briefly described elsewhere (Gregory, 1936, 1937, 1939*b*).

Economic losses from leaf diseases

Direct loss of crop follows when flowers are blemished by spore infections. Flower spotting may follow invasion of the perianth by *Sclerotinia polyblastis*, *Stagonospora Curtisii*, and *Botrytis narcissicola*, while flower stalks of Poeticus varieties of narcissus are sometimes rotted by *Ramularia vallisumbrosae*. Indirect loss of crop occurs through weakening of the plant by premature destruction of leaf tissue. The period during which the narcissus is in leaf is shortened by two or three weeks at the end of the growing season so that the starch reserve of the bulb decreases. The narcissus, like certain other spring flowering bulbs, forms its flowers during the summer and the development of the flower in spring consists in an extension of cells already formed. Reduced reserves at the end of each leafage period affects the number of flower buds to be laid down and reduces the flower crop in the following year. The performance of a stock of bulbs is influenced by its history and experimental plots must be studied over a period of years before conclusions can be arrived at on factors influencing cropping. The studies here recorded were planned to find methods of controlling leaf diseases and to measure the indirect loss of crop as expressed in bulb increase in weight, number, grade and flowering capacity. The account is based on observations of the control of disease obtained by spraying with various fungicides, and on some crop records carried out on a commercial scale. Detailed crop records on small experimental plots will be given in later papers.

Preliminary testing of fungicides

Numerous fungicides were tested in the winters of 1935–6 on commercial stocks of narcissus at different stages of growth. Data presented in this paper refer mainly to *Narcissus pseudo-narcissus* var. "Golden Spur", a widely grown Yellow Trumpet daffodil susceptible to white mould and fire. Plantings in their second or third flowering season were

[1] Now attached to the Ministry of Agriculture and Fisheries, Plant Pathological Laboratory, Milton Road, Harpenden, Herts.

usually selected because these diseases are rarely severe in the first season after planting. The preliminary field testing of fungicides was assessed by eye judgement, supplemented at times by counts of infected leaves and lesions. Counts were discontinued when it became apparent that Bordeaux mixture gave nearly perfect control of white mould and good control of fire.

Tests were carried out at eleven centres in west Cornwall and the Isles of Scilly. The sprays were usually applied with a pneumatic knapsack sprayer at 50–75 lb. pressure, but in a few tests a bucket pump, headland sprayer and wheelbarrow sprayer were also used. Dry dusts were applied with either a rotary blower or a bellows knapsack. With most of the wet sprays one of several proprietary spreaders was used. To wet the glaucous leaf of narcissus it was necessary to use a higher concentration of wetting agent than normally recommended by the manufacturers but the quantity required varied considerably according to the age of leaf and variety.

In these tests spraying was usually begun when the leaves were about 4–6 in. high and was continued at approximately monthly intervals. The size of the plot used varied from 15 to 400 sq. yd. for each treatment, but it was found that a square plot six beds wide and 10 yd. long, with an area of about 1/50 acre was convenient for spraying and eye observation. The disease which appeared most commonly was white mould, sometimes fire was also present, and occasionally fire appeared alone.The results of tests with various sprays are summarized below.

Sulphur. Sulphur in various forms had little effect in controlling infection. Sulphur dust applied under optimum conditions did not visibly prevent the secondary spread of white mould, and compared unfavourably with an adjacent test with copper lime dust.

Lime sulphur of S.G. 1·3 at 4 %, a strength which caused some burning of the expanded perianth, was followed at various centres by a substantial reduction in the number of primary white mould lesions. Repeated applications, however, failed to prevent the secondary spread of white mould and fire, and towards the end of the season sprayed and unsprayed plots were usually indistinguishable. A similar early advantage, not subsequently maintained, was obtained with a proprietary colloidal sulphur preparation and with two samples of Newton's (1930) potash sulphur resinate. Two organic sulphur preparations failed to give any visible control of white mould or fire.

The behaviour of sulphur is interpreted as showing that under the cool conditions in which this work was carried out (Scilly Isles: mean daily maximum temperature Feb. 50° F., Mar. 52° F.) sulphur is only mildly active against these fungi. Primary infections of *Ramularia vallisumbrosae* were reduced in number by from one-fifth to one-half on the sprayed plots, but in the stage of secondary spread this reduction may not be sufficient to prevent enough lesions developing to kill the leaves.

Tests with sulphur fungicides clearly showed no practical value against white mould and no effect was observed on the incidence of fire. Very heavy applications of lime sulphur or colloidal sulphur to the young foliage reduced the number of primary white mould lesions, but this process was never complete. After the first two years, tests with sulphur fungicides were almost discontinued on account of the superiority of copper fungicides.

Copper. Bordeaux mixture. The spray was prepared with hydrated lime according to the formula 4–4–40 or 4–3–40 and, when started early, it gave practically complete control of white mould and satisfactory control of fire. One application proved superior to two of a proprietary copper oxychloride preparation. Failures resulted when fresh samples of lime were not used, but in no experiment did any other spray tested equal the control given by Bordeaux mixture.

In each of the years 1935–9 Bordeaux mixture stood out pre-eminently over other fungicides tested, including cuprous oxide and copper oxychloride wet sprays, which however approached it in efficiency. This superiority was substantiated where it was possible to compare effects on the flower counts. The efficiency of Bordeaux mixture used in winter during a season of high rainfall in districts exposed to gales no doubt resides in the adhesiveness of the dried deposit. This adhesiveness was illustrated by an application to foliage of the variety "Soleil d'Or" in mid-December 1936. In the third week in March 1937 after a period of abnormally high rainfall in the Isles of Scilly traces on the foliage were still easily visible.

Bordeaux mixture not only prevents primary infections of the leaf tips from sclerotia on the ground, and checks secondary spread if the primary lesions have already developed, but it also prevents the development of sclerotia of *Ramularia vallisumbrosae* and *Sclerotinia polyblastis* on the leaves and thus indirectly reduces the risk of the soil becoming contaminated by these fungi. Under optimum condi-

tions second and third year plantings sprayed with Bordeaux mixture may retain the foliage for as much as three weeks longer than the untreated plots.

Burgundy mixture (4–5–40) gave nearly as good control of white mould and fire as did Bordeaux mixture on the same field, but its use was discontinued on account of severe scorching of leaf tissue.

Cuprous oxide suspensions, which were tested extensively because they are easily prepared and leave an inconspicuous deposit, have shown considerable promise. Several preparations including both red and yellow forms of cuprous oxide were employed at concentrations varying from 0·125 to 0·28 % metallic copper. At all concentrations under certain conditions copper injury was noted on "Golden Spur" no less severe than with Bordeaux mixture. Control of white mould and fire so far obtained have been inferior to Bordeaux mixture but good enough to keep a proportion of the foliage in active condition when the untreated plots were completely withered, and also to prevent the development of sclerotia on the sprayed leaves. There is evidence that washing off of the copper from the foliage was facilitated by the large quantities of wetting agent used to obtain cover.

Cupric oxide suspensions at concentrations of 0·25 and 0·5 % metallic copper gave fairly good control of white mould and fire but severely scorched the leaves.

A proprietary copper oxychloride gave good control of both diseases but spray damage resulted from concentrations as low as 0·04 % copper under conditions in the Isles of Scilly. Ammoniacal copper carbonate containing 0·03 % copper and basic copper acetate with 0·1 % copper failed to reduce the secondary spread of white mould when tested on a plot where primary lesions had already developed, probably because of poor retention of this spray. Five other experimental copper sprays were also tested but were discarded on account of spray damage or because they were less effective than Bordeaux mixture and cuprous oxide.

Copper injury occurred on a number of plots and narcissus is apparently sensitive to damage by both copper and lime. The effect of severe scorching of the leaves from copper sprays on the subsequent crop is dealt with below. The first sign of injury is a purplish brown mark on the epidermis especially near the tips of the leaves. This is similar in appearance to the purplish marking produced by some other causes, such as abortive leaf scorch infection. At a later stage a white somewhat sunken spot appears, often near the middle of a chocolate one and where cupping of the leaf retains the spray. In warm dry weather copper spraying may be followed by considerable scorching of the leaf tips. Another possible deleterious effect from spraying with Bordeaux mixture is a retardation in development of the plants during the ensuing season.

Copper dusts. Owing to drifting of the dusts in the wind larger plots were required for testing dusts than for wet sprays. Dusts tested included two proprietary copper-lime dusts, cuprous oxide with various diluents, and cupric oxide with lime. They were applied at the rate of about 1 cwt./acre and nearly every sample tested was of some use in protecting the foliage in comparison with untreated plots.

The most satisfactory control was obtained with a cuprous oxide-lime dust containing 20 % copper, but the control obtained never approached the efficiency of wet sprays such as Bordeaux mixture and cuprous oxide suspensions even when five times the weight of copper per acre normally used in a wet spray was applied as dust.

Other organic sprays. Shirlan A.G. at 1·25 % in one, two or three applications and at 2·5 % in one application was non-phytocidal and reduced the number of lesions in a manner comparable with lime sulphur, but inferior to that obtained with copper dusts, which are, of course, easier to apply.

The effect of spraying on crop yield

As soon as it became apparent that control of white mould could be obtained with Bordeaux mixture experiments were set up to investigate the effect of such control on subsequent bulb and flower cropping.

Effect on bulb crop. A large scale unreplicated test was begun in the Truro area on a commercial stock of "Golden Spur" involving approximately 2 tons of bulbs planted after broccoli in a two-year rotation. The bulbs had been roughly graded as "flowering-size", given a routine hot-water treatment for 3 hr. at 110° F. and planted in September 1935.

A number of beds were bisected transversely by a path in the manner indicated diagrammatically in Table 1. For purposes of treatment and recording the block of beds was divided into six plots, three below the path, and three others above the path corresponding to the plots below the path and consisting of the upper halves of the same beds. The total length of the beds in each plot is also shown in Table 1. One plot below the path was sprayed with 4–4–40 Bordeaux mixture three times during each of the seasons 1936 and 1937, while the remainder of the plots were not treated.

In 1936 spray was applied on 26 January, 5 March and 7 April. In this season, the first after planting, the foliage on the unsprayed plots went down early in May with an attack of fire but the sprayed foliage remained green for another 2 or 3 weeks and then ripened off normally. In 1937 spray was applied on 9 March, 18 March and 9 April. In the middle of April the unsprayed plots began to be killed by an epidemic of white mould, and were almost completely withered by 7 May, while the sprayed plots ripened off normally in the last week of May. During the two seasons the plants in sprayed plots retained their foliage for a total of about 6 weeks longer than the untreated plots.

Lifting was carried out in the first week in June 1937 in ideal weather. The bulbs were roughly cleaned and the produce of each half bed weighed separately on the field, though it was obvious on lifting that the produce of the sprayed half beds consisted of heavier and larger bulbs. Table 1 shows the crop from each plot expressed in terms of the average weight of bulbs lifted per 1 ft. run of bed (actual weights lifted are affected by the variation in lengths of beds referred to above).

TABLE 1. *Weight of bulbs from sprayed and unsprayed plots*

	Lower halves of beds				Upper halves of beds		
Bed nos.	Total length of beds ft.	Wt. lifted 1937 lb.	Average wt. per 1 ft. bed lb.		Total length of beds ft.	Wt. lifted 1937 lb.	Average per 1 ft. bed lb.
2– 8	351	589	1·68		638	968	1·52
9–28	1551	3531 (sprayed)	2·28	Path	1807	2754	1·52
29–43	1562	2688	1·73		1359	2107	1·55

As Table 1 shows, the sprayed plot gave a yield of 2·28 lb. per 1 ft. run of bed while the highest yielding unsprayed plot gave only 1·73 lb. The average yield for all unsprayed plots was 1·59 lb. Thus the yield from the sprayed plot was about 43 % heavier than the average yield of the unsprayed.

The weights given above were recorded on the day of lifting. It was thought that the sprayed bulbs, having ripened later, might have lost less water than the unsprayed, or that the relative weights from different plots after a period of drying in store might change substantially, but this is not so. It was not possible to weigh the produce of all the beds a second time, but certain beds were marked out for further study, and the bulbs from the sprayed and unsprayed halves of one of these beds were weighed twice more during the summer. Table 2 shows that the percentage weight lost by each lot of bulbs during the period in store is practically identical, so it is evident that the relative weights at lifting or at any other time are a trustworthy guide to the effect of treatments on bulb weight. This conclusion has been confirmed by later experiments.

Data on increase in net weight of bulbs lifted still leave much information to be obtained. We need to know how the treatment has affected both number of bulbs and weight of individual bulbs. On an experiment of this size detailed information over the whole stock

TABLE 2. *Change of weight of bulbs during storage*

	1 June (lifted)		11 June (cleaned)		6 Sept. (replanted)
Unsprayed (90 ft.)	143 lb.		93 lb.		89 lb.
% loss in interval		34·9		6·3	
Sprayed (61 ft.)	129 lb.		84 lb.		76 lb.
% loss in interval		34·8		6·2	

was not obtainable, but a sample was investigated. The bulbs from both sprayed and unsprayed halves of the above bed were graded, counted and weighed at the time of cleaning. The data obtained may be expressed in terms familiar to bulb growers as follows (Table 3).

TABLE 3

Treatment	Yield per 1 ft. bed lb.	No. bulbs per 1 ft. bed	No. bulbs lifted	Wt. bulbs lifted lb.	Wt. per 1000 lb.
Sprayed	2·11	21·7	1323	84	63·5
Unsprayed	1·59	22·3	2005	93	46·5
Ratio: Sprayed / Unsprayed	1·33 / 1				1·37 / 1

The ratio of yields of sprayed and unsprayed beds (1·33) and the ratio of weights of individual bulbs (expressed above as weight per 1000, 1·37) were practically identical, so it is evident that during the 2 years in which spraying was carried out the increased yield consisted of a larger average size of the individual bulbs, and not of any increase in the number of bulbs lifted. The data provide an independent check on this conclusion because the number of bulbs lifted per 1 ft. was almost identical on the sprayed and unsprayed half of the bed (22·3 and 21·7 bulbs lifted per ft. respectively). Spraying had thus improved the weight within the grade but had not increased the number of bulbs.

The possibility that retention of the sprayed foliage for a longer period might give extra opportunity for infestation by bulb fly was considered and, on 28 July, 400 bulbs, consisting of four samples from sprayed beds and four from the corresponding unsprayed half of the same beds were examined by Mr L. N. Staniland. Infestation was found to be 6·0% in sprayed and 7·5% in unsprayed bulbs, thus showing no evidence of any difference between the two treatments.

Effect on flower crop. Part of the above stock of "Golden Spur" was investigated for a further period of 2 years to compare the flower crop from sprayed and unsprayed bulbs and the effect of further treatments. Progeny from three of the beds was replanted in September 1937 in one strip from end to end of the field, but separated by transverse paths into six plots. In the following season the number of plants and flowers was counted on three rows of each of the six plots and it was found (Table 4) that the bulbs sprayed 1936–7 gave a crop averaging 63·2% (i.e. 63·2 flowers per 100 bulbs planted) while the unsprayed plots gave only 43·7%. Bulbs previously sprayed, which had individually averaged 37% heavier than the unsprayed plots, gave 41% more flowers than did bulbs from the unsprayed half of the same bed. On 8 February and 8 April 1938, treatments with copper fungicides were continued on the plots previously sprayed, by applying an aqueous suspension of cuprous

oxide with a wetting agent at a concentration of 0·125 % metallic copper and the same spray was used again in 1939, on 14 March and 15 May.

TABLE 4. *Treatments and flower crop from sprayed and unsprayed plants*

	Treatments	
	Unsprayed plots	Sprayed plots
1935	Planted	Planted
1936	Heavy infection by fire	Protected by Bordeaux mixture
1937	Heavy infection by white mould Lifted and replanted	Protected by Bordeaux mixture Lifted and replanted
1938	Remained healthy	Damaged by cuprous oxide spray
1939	Heavy infection by fire Lifted and replanted	Some control of fire by cuprous oxide Lifted and replanted

	Unsprayed plots						Sprayed plots					
	Yield 1937		No. of flowers*				Yield 1937		No. of flowers*			
Plots	in lb.	No. of bulbs	1938	%	1939	%	in lb.	No. of bulbs	1938	%	1939	%
1–2	1·47	648	252	39	775	120	2·24	778	462	59	846	104
3–4	1·59	751	361	48	906	121	2·11	687	469	68	812	119
5–6	1·67	810	356	44	842	104	2·17	647	401	62	741	115
Total		2209	969		2523			2112	1332		2399	
Average				43·7		114·2				63·2		113·6

* i.e. number of flowers per 1000 bulbs planted.

The spring of 1938 was warm and dry and no epidemic foliage disease appeared on the unsprayed plots in this test, but by 22 April a marked scorching effect of the leaf tips and down the leaf margins of the sprayed leaves was evident. About a quarter of the leaf surface appeared variously discoloured or scorched while the unsprayed plots remained green and healthy. The damage did not become worse, and the foliage of both sprayed and unsprayed plants matured together during the third week in May. This offered an opportunity to test the effect of damage on the subsequent flower crop under conditions uncomplicated by the effect of simultaneously controlling a foliage disease. Flower counts made on the same plants in 1939 (Table 4) show that crops from the sprayed (damaged 1938) and unsprayed (normal 1938) plots were practically identical. It is possible that on the spray-damaged plots a residual increase from disease control over the period 1936–7 might have counterbalanced a crop reduction from spray damage in 1938, but it is unlikely that the two effects would balance so closely. Probably the early loss of a small proportion of the leaf is of little importance in comparison with the loss of the whole leaf from disease. (Confirmatory evidence that spray damage did not adversely affect cropping of narcissus "Sunrise" is given below.)

In 1939 spray damage was negligible. During May a heavy infection of fire occurred from foci on numerous flowers of rogue varieties left unpicked to wither on the plant. The unsprayed beds were attacked earlier, but the foliage of sprayed and unsprayed plots died down more or less at the same time during May, and the control given by the cuprous oxide preparation used was poor. The bulbs were lifted in June 1939 and, as shown in Table 5, the average increase from sprayed and unsprayed plots was identical.

TABLE 5. *History of bulb weights in the six plots over the four-year period* 1935–9

	Unsprayed					Sprayed				
Plot	Wt. before planting 1935 (estimated)	Wt. on lifting 1937	% increase	Wt. on lifting 1939	% increase	Wt. before planting 1935 (estimated)	Wt. on lifting 1937	% increase	Wt. on lifting 1939	% increase
1	80 lb.	132 lb.	65	298 lb.	126	55 lb.	132 lb.	140	261 lb.	98
2	80	143	79	311	117	57	129	126	273	112
3	80	150	87	311	107	60	139	132	325	134
Average			77		117			133		115

To summarize the work on this stock it is evident that good control of foliage diseases during 1936 and 1937 by Bordeaux mixture resulted in nearly doubling the rate of bulb increase. Cuprous oxide in 1938–9 did not increase bulb or flower crop.

Increase of stock following a single late application of Bordeaux mixture. In 1935 the bulbs of a stock of "Golden Spur" in the Penzance area were divided into two lots each weighing 2079 lb. These were planted side by side in two blocks of five beds. No spray was applied in 1936 and as often happens in the first year after planting neither block suffered much foliage infection. By 30 March 1937 an attack of white mould was developing, and to one plot of about $\frac{1}{4}$ acre 40 gal. of 4–4–40 Bordeaux mixture was applied without a wetting agent. This single late application gave surprisingly good control of white mould as judged by eye observations during April and May. In June of the same year the stock was lifted and, as shown in Table 6, after preliminary drying the yield of bulbs from the sprayed plot was greater by 13% than that from the unsprayed.

TABLE 6. *Yields from plots with one late application of Bordeaux mixture*

	Unsprayed	Sprayed
Weight planted 1935 (lb.)	2079	2079
Weight lifted 1937 (lb.)	4320	4880
Ratio of yields	1	1·13
% increase on weight planted	108	135

A comparable increase as judged by flower count was obtained on a small test on "Golden Spur" in the Truro area. One of two small second-year beds was sprayed on 8 April 1938 with 4–4–40 Bordeaux mixture with a wetter. No spray damage was noted, and little disease appeared on the unsprayed bed until shortly before the foliage ripened off when the leaf bases of the sprayed plants remained green for a time. In spring 1939 bulb and flower counts were made on these beds (Table 7).

TABLE 7

	Unsprayed	Sprayed
No. of bulbs	1175	1085
No. of flowers	1763	1825
% flowering	150	168

Effect of Bordeaux mixture in absence of disease. A stimulatory effect of copper on the growth of certain plants has been recorded by some writers, and the increases observed in these experiments on narcissus might be regarded as indirect effects of this nature, rather than a results of disease control. This is negatived however by an experiment on narcissus "Cheerfulness" sprayed with Bordeaux mixture during the dry spring of 1938 when neither spray injury nor foliage diseases appeared on the plots. The beds had been planted in 1937 with small bulbs graded as "chips" of average weight 34 lb. per 1000. Five beds were sprayed on 7 April 1938 with 4–4–40 Bordeaux mixture with a wetter. Flower counts were made in 1938 before spraying was begun and again in 1939 and, as shown in Table 8, the crop from the treated and untreated beds agree closely (104·6 and 100·0% flowering). Evidently on the variety "Cheerfulness" one relatively late application of Bordeaux mixture had no intrinsic effect in increasing the crop in the absence of leaf diseases.

TABLE 8. *Flower crop of "Cheerfulness" following Bordeaux mixture without injury or foliage disease*

Unsprayed 1938				Sprayed 1938			
Bed no.	No. of bulbs	No. of flowers 1938	No. of flowers 1939	Bed no.	No. of bulbs	No. of flowers 1938	No. of flowers 1939
1	701	71	729	2	773	70	815
4	765	69	777	3	779	58	846
5	757	46	795	6	784	119	749
8	764	67	838	7	817	72	840
9	756	82	779	10	809	153	719
Totals	3743	335	3918		3962	472	3969
% flowers		9	104·6			12	100·0

Effect of spray injury. In an experiment with "Golden Spur" described above no evidence was found that crop reduction followed a severe scorching of leaf tissue in the warm spring of 1938. The interpretation of this experiment was complicated by previous spraying treatments, and a reduction of crop, if it existed, might have been obscured by a residual increase due to spraying in previous years. In the same season, however, similar spray injury followed the application of Bordeaux mixture to the variety "Sunrise".

In 1937 sixteen beds of Barrii "Sunrise" were planted for experimental purposes in the Lizard area of Cornwall. The beds were taken in pairs and one of each pair at random chosen for spraying or to be left unsprayed. In 1938 before any treatments were begun the flowers on a row of 300 bulbs in corresponding positions on each bed were counted as a test of the uniformity of the material and, as shown in Table 9, no significant difference was noted between the cropping of the two lots of beds. The whole of each bed chosen for spraying was treated on 1 April 1938 with 4–4–40 Bordeaux mixture. No foliage diseases appeared subsequently, but the sprayed plots soon showed considerable scorching which was not visible on unsprayed beds; this appearance was retained to the end of the season when both lots of beds ripened off together. In 1939 flower counts were made on the same sample of 300 bulbs in each bed used for the 1938 records. In 1938 before treatments were begun, the beds designated "unsprayed" had produced 3·62 % more flowers than the beds to be "sprayed", but in 1939 they produced only 2·65 % more flowers than the "sprayed" plots. Table 9 shows that the differences in flower count between the two lots of beds were not significant in either 1938 or 1939, and it is concluded that in "Sunrise" fairly severe spray damage has no appreciable adverse effect on flower crop in the following year.

TABLE 9. *Cropping of "Sunrise" before and after Bordeaux injury*

	No. flowers 1938 per 300 bulbs			No. flowers 1939 per 300 bulbs		
Bed nos.	Unsprayed	Sprayed	Diff.	Unsprayed	Sprayed	Diff.
1– 2	253	213	+40	615	542	+73
3– 4	240	239	+ 1	505	478	+27
5– 6	228	218	+10	516	495	+21
7– 8	230	228	+ 2	578	522	+56
9–10	240	233	+ 7	626	607	+19
11–12	209	210	− 1	629	578	+51
13–14	217	211	+ 6	581	630	−49
15–16	213	214	− 1	518	598	−80
Total	1830	1766	64	4568	4450	118
	$x=8$			$x=14·75$		
	S.E. of diff. $=4·78$			S.E. of diff. $=18·75$		
	$t=1·673$			$t=0·7875$		

Retardation in the year following spraying

One unexpected effect sometimes observed in the year following the spraying of "Golden Spur" with Bordeaux mixture was a small but definite retardation in date of emergence of the leaves in spring, and in the subsequent date of flowering. This effect, which will be discussed in more detail in a subsequent paper, was first noted in the spring of 1937 at Truro, and was seen again at the same centre in 1938 and 1939 but to a smaller extent. The possibility to be borne in mind is that in an early flower district, when the price is falling, a comparatively slight retardation involving the loss of one or two markets might offset to some extent the gain in crop from spraying. This retardation has not been observed in varieties other than "Golden Spur".

Dates of application

The most satisfactory control of narcissus white mould and fire has been obtained with three applications of 4–4–40 or 4–3–40 Bordeaux mixture, beginning when the shoots are 4–6 in. high, and repeated at monthly intervals. This treatment gave excellent results on second and third-year plantings that had received no treatment in the first year, and under conditions favourable to epidemic attacks. It is probable that in normal seasons a single application made shortly after flowering would suffice to keep the stock reasonably free from disease, and even this might be omitted the season after planting, when leaf diseases are rarely of importance. An additional earlier application in the second year would then be advisable, however, to prevent primary infection of the leaves by white mould. If second-year beds are not sprayed three applications may be necessary in the third year for complete control. In general a single late application on the mature foliage is of more benefit than a single early one applied to the leaf tips only.

The beneficial effect of hygienic measures has been demonstrated. Raking off and burning dead leaves that carry the resting stages of *Ramularia vallisumbrosae* and *Sclerotinia polyblastis* (Gregory, 1938, 1939*a*) helps to control the disease, but can never be done thoroughly enough to replace spraying, and attempts to kill the sclerotia by surface treatment on the soil before germination have proved fruitless. Removal and destruction of all spotted flowers, rogue flowers and late flowers which open after the main crop has been picked is highly important. Narcissus flowers in the open are particularly susceptible to ascospore infection by *Sclerotinia polyblastis* and when left on the plant they form reservoirs of conidia from which epidemics on the leaves arise later in the season.

Summary

1. Spraying experiments on *Narcissus pseudonarcissus* "Golden Spur", a variety susceptible both to white mould (*Ramularia vallisumbrosae*) and fire (*Sclerotinia polyblastis*) in the south-west of England, were carried out to find effective control measures and to measure the effect of these foliage diseases on bulb and flower crops.

2. Judged by eye observation Bordeaux mixture gave excellent control of white mould and good control of fire. Cuprous oxide suspensions were also fairly effective. Sulphur fungicides reduced the number of primary lesions but were markedly inferior to copper sprays and dusts in controlling secondary spread.

3. Crop records indicate that control of epidemic foliage diseases by Bordeaux mixture is followed by substantial increases in the average size and flowering capacity of bulbs lifted but not in the number of bulbs lifted.

4. The application of Bordeaux mixture to the variety "Cheerfulness" in the absence of disease on the untreated healthy plots did not lead to a relative increase in crop.

5. Narcissus foliage is liable to damage from copper sprays under certain conditions. Tests on the varieties "Golden Spur" and "Sunrise" indicated that such damage did not significantly reduce subsequent flower crop.

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STUDIES ON THE FEEDING METHODS AND PENETRATION RATES OF *MYZUS PERSICAE* SULZ., *MYZUS CIRCUMFLEXUS* BUCKT., AND *MACROSIPHUM GEI* KOCH.

By F. M. ROBERTS

Rothamsted Experimental Station, Harpenden, Herts

With Plates 14 and 15

EXPERIMENTS on the transmission of three strains of *Hyoscyamus* virus III, potato virus *Y*, and cucumber virus I by the aphis species *Myzus persicae* Sulz., *M. circumflexus* Buckt., and *Macrosiphum gei* Koch. (Watson, 1938; Watson & Roberts, 1939) have shown that most infections are obtained after previously fasted aphides have fed for only 2 min. on the source of infection. Infectivity decreases rapidly when the infection feeding time is increased to 15 min. and still further to 3–5 hr.

Though this response to short infection feeding periods is common to the three aphides with the viruses under discussion there are slight variations in the efficiency of the different aphis species with some of the viruses. It was hoped, therefore, that a study of the feeding habits and penetration rates of these aphides on tobacco might throw light on the cause of the variations.

No decrease in the numbers of infections with increased feeding periods of *Myzus persicae* such as are described above were obtained with Sugar Beet Yellow virus. The feeding methods of this aphis on sugar beet, therefore, were also investigated to see whether any explanation could be found for the different relationship between insect and virus observed for Sugar Beet Yellows and viruses of the *Hyoscyamus* III type.

MATERIAL AND METHODS

The aphides used were from cultures of *Myzus persicae*, *M. circumflexus*, and *Macrosiphum gei* kept in stock as partheno-genetic, apterous, viviparous females. They were from the same stock as those used in the insect transmitting experiments (Watson & Roberts, 1939). The plants used were seedlings of White Burley tobacco, and Kleinwanzleben E sugar beet.

Aphides, previously collected and starved were placed in batches of about thirty on young leaves supported in wet sand under a bell jar to ensure humid and undisturbed feeding conditions. After the required feeding time the leaves were plunged into a shallow dish containing boiling Schaudinn's fluid. After a few seconds they were transferred to absolute alcohol where the sections of leaf to which aphides still adhered were cut out in sizes convenient for embedding. These sections were fixed for ½ hr. in Carnoy's fluid, washed in a solution of iodine in absolute alcohol, and again in fresh absolute alcohol, and transferred by gradual stages through cedar-wood oil to paraffin wax with a melting point of 56°.

Although many aphides were lost by this method, especially in the Schaudinn's fluid, no other was found which gave equally rapid and satisfactory fixation for both insects and leaf tissue. Loss of aphides took place chiefly in the short feeding periods; for example, out of 164 *Myzus circumflexus* fed for 5 min. on tobacco leaves, forty were finally embedded in wax, and of these six gave stained and mounted preparations showing the stylets in place in the tissues. In the short feedings of the aphis *M. persicae*, which had not previously been starved, the percentage of success was even less. In one

batch of approximately 370 aphids fixed, fifty-eight were embedded in wax, and only four successful mounted preparations were obtained.

Most of the sections were cut at 20 μ, as thinner sectioning rarely gave the complete path taken by the stylets in one or two serial sections. The stylets were often broken and moved in cutting and a more accurate indication of the depth they had penetrated was given by the stylet tracks. Measurements were made only where the stylet track was clearly visible, and were taken from the point of entry of the stylet to the end of the stylet track. Curves were disregarded, so in some cases the figures given in the tables are not the actual length of stylet in the leaf tissue.

Sections were stained in safranin and light green, both in 95 % alcohol, and mounted in canada balsam.

Some of the data included here on the penetration rates of *M. persicae* on tobacco are from preparations made by Mrs W. Cochran (Mitchell, 1937) in which a different fixing technique was used. By this method boiling absolute alcohol was poured over the aphides feeding on leaves in shallow dishes. The leaves with the insects attached were then cut up, further fixed in fresh absolute alcohol, transferred gradually to cedar wood oil, and embedded.

Feeding habits and penetration rates of aphides

(1) Myzus persicae *on tobacco*

Aphides were fixed on tobacco at feeding periods of 5, 15, and 60 min. The majority had previously been starved, though in Table 1 data are included on some previously unstarved insects in the 5 min. feeding period. Previous experiments showed (Watson & Roberts, 1939) that for all aphides and viruses infectivity increased considerably with increasing time of preliminary fasting.

Reference has previously been made to the difficulty of fixing aphides in the unstarved group, possibly because the latter take longer to "settle" on the leaves than the fasting aphides. Data on their penetration rates, therefore, are scanty. In Table 1, however, comparison between four unstarved and four starved aphides (*M. persicae*) shows that the average depth of penetration is slightly more for the starved insects. This difference, however (between 45 and 52 μ), is not thought to have any bearing upon the difference in infectivity between the starved and unstarved insects. The average depth penetrated by the four starved and four unstarved aphides is 49 μ.

The results obtained by Mitchell (1937) for 5 min. feeding periods gave a deeper average penetration of 63 μ. For purposes of comparison with the short feeding periods of *M. circumflexus*, therefore, the average depth of penetration from my own preparations only will be taken into consideration in order to ensure uniformity in the method of measurement.

Table 1 shows that none of the fifteen aphides fed for 5 min. had penetrated as far as the phloem though two had reached cells adjoining it. The great majority had penetrated through the epidermis usually only as far as the first or sometimes the second cell of the mesophyll (Pl. 14, figs. 1, 2). Even in the 15 min. feedings only one insect reached the phloem, and one the cells adjoining it. After 1 hr. feeding five out of nine, or 55 %, of the aphides were feeding in the phloem, and two of the remaining four were near it. Thus at the period when the highest number of successful infections are obtained, i.e. after 5 min. on the infected plant, the majority of the aphides have penetrated only just below the epidermis.

59 % of the penetrations by *M. persicae* were intracellular. 28 % were intercellular and of these only one had penetrated through a stoma (Pl. 14, fig. 1).

TABLE 1. *Method of feeding and penetration rates of* Myzus persicae *on tobacco*

Feeding time	Slide no.	Method of penetration: Intra-cellular	Inter-cellular	Doubtful	In phloem	Not in phloem	Depth of penetration (μ)
5 min. (unstarved aphides)	1	+	.	.	.	+	46
	2	.	.	+	.	+	44
	3	+	.	.	.	+	56
	5	.	+*s*	.	.	+	36
Total	4	2	1	1	0	4	Mean 46
5 min. (starved aphides)	1	+	.	.	.	+*c*	90
	2	+	.	.	.	+	38
	3	+	.	.	.	+	54
	4	.	+	.	.	+	24
Total	4	3	1	0	0	4	Mean 52
5 min. (starved aphides)	*1	+	.	.	.	+*c*	100
	*2	.	+	.	.	+	11
	*3	+	.	.	.	+	66
	*4	.	+	.	.	+	44
	*6	+	.	.	.	+	144
	*8	+	.	.	.	+	42
	*9	+	.	.	.	+	36
Total	7	5	2	0	0	7	Mean 63
Total for all 5 min. feedings	15	10	4	1	0	15	
%		67	27	7	0	100	
15 min.	*10	+	.	.	.	+	42
	*11	.	+	.	.	+	56
	*12	+	.	.	.	+	6
	*13	+	.	.	.	+	156
	*14	+	.	.	.	+*c*	96
	*15	.	+	.	.	+	112
	*16	+	.	.	+	.	102
	*17	+	.	.	.	+	32
Total	8	6	2	0	1	7	Mean 75
%		75	25	0	13	88	
1 hr.	*18	.	+	.	.	+	60
	*19	.	.	+	.	+*c*	96
	*20	.	.	+	+	.	104
	*21	+	.	.	+	.	206
	*22	.	+	.	+	.	200
	*23	+	.	.	.	+*c*	90
	*24	.	.	+	+	.	210
	*25	+	.	.	.	+*c*	142
	*26	.	+	.	+	.	176
Total	9	3	3	3	5	4	Mean 143
%		33	33	33	56	44	
Total	32	19	9	4	5	27	
%		59	28	13			

* From Mitchell (1937); *c* near phloem; *s* penetration through stoma. N.B. Measurements in first eight slides made in straight line from point of entry of stylets to end of stylet track.

(2) Myzus circumflexus *on tobacco*

Aphides were fixed feeding on tobacco after the following periods: 5 min. and 1, 1½, and 24 hr.

The average depth of leaf penetrated by this insect (Table 2) after 5 min. feeding was 57 μ compared with 49 μ by *M. persicae* (Pl. 14, figs. 3, 4*a* and 4*b*). None of the short period feedings was successful in reaching the phloem. Only one out of fifteen aphides had penetrated as far as the centre of the leaf and this was not in the vicinity of a vascular bundle.

TABLE 2. *Method of feeding and penetration rates of* Myzus circumflexus *on tobacco*

Feeding time	Slide no.	Method of penetration: Intra-cellular	Inter-cellular	Doubtful	In phloem	Not in phloem	Depth of penetration μ
5 min.	1	+	.	.	.	+*d*	110
	2	.	+*s*	.	.	+	26
	3	.	.	+	.	+	.
	4	.	+	.	.	+	60
	5	.	+	.	.	+	62
	6	.	.	+	.	+	60
	7	.	+	.	.	+	30
	8	.	+	.	.	+	40
	9	.	.	+	.	+	.
	10	.	+	.	.	+	54
	11	.	+*s*	.	.	+	26
	12	.	+	.	.	+	110
	13	.	+*s*	.	.	+	54
	14	.	.	+	.	+	.
	15	.	+	.	.	+	50
Total	15	1	10	4	0	15	Mean 57
%		7	67	27	0	100	
1 hr.	1	.	.	+	+	.	110
	2	.	+	.	+	.	112
	3	+*s*	.	.	+	.	90
1½ hr.	1	.	.	+	+	.	.
	2	.	.	+	+	.	.
	3	.	+	.	+	.	110
Total	6	1	2	3	6	0	Mean 105
%		17	33	50	100	0	
24 hr.	1	.	+	.	+	.	102
	2A	+	.	.	+	.	180
	2B	.	+	.	.	+	146
	3	.	+	.	.	+	190
	4	.	+	.	+	.	170
	5	+	.	.	+*b*	.	126
	6A	.	+	.	.	+*c*	138
	6B	.	+	.	.	+	44
	7	.	+	.	+	.	190
	8	.	+	.	+	.	196
Total	10	2	8	0	6	4	Mean 147
%		20	80	0	60	40	
Total	31	4	20	7			
%		13	64	23			

b In xylem; *c* near phloem; *d* deep enough, but not in vicinity of vascular bundle; *s* penetrated through stoma.

In the 1–1½ hr. feeding group only six aphides were fixed and all had succeeded in reaching the phloem. In the 24 hr. group 60% were feeding in the phloem; this group includes one aphis which had withdrawn its stylets from the phloem, and was feeding in the xylem (Pl. 14, figs. 5, 6). The remainder had, for the most part, penetrated the sufficiently deeply, but not in the vicinity of a vascular bundle.

The most striking difference between *M. circumflexus* and *M. persicae* lies in the fact 64% of the penetrations by the former were intercellular (Table 5). 13% were intracellu and 23% doubtful (Pl. 14, figs. 4, 5). Penetration through the stomata also appears tc more frequent than with *M. persicae* (Table 5).

(3) Macrosiphum gei *on tobacco*

Aphides were fixed at feeding periods of 1 and 24 hr. No stomatal penetrations v observed (Table III).

TABLE 3. *Method of feeding and penetration rates of* Macrosiphum gei *on tobacco*

Feeding time	Slide no.	Method of penetration: Intra-cellular	Inter-cellular	Doubt-ful	In phloem	Not in phloem	Depth of penetration μ
1 hr.	2	+	.	.	+	.	80
	3	+	.	.	+	.	84
	4	+	.	.	+	.	120
	5	+	.	.	+	.	120
	6A	+	.	.	.	+	50
	6B	+	.	.	.	+	56
	7	+	.	.	+	.	66
	8	+	.	.	+	.	130
Total	8	8	0	0	6	2	Mean 88
%		100	0	0	75	25	
24 hr.	1	+	.	.	.	+*d*	104
	2A	+	.	.	+	.	160
	2B	+	.	.	+	.	160
	2C	+	.	.	+	.	164
	3	+	.	.	+	.	140
	4	+	.	.	+	.	210
Total	6	6	0	0	5	1	Mean 156
%		100	0	0	83	17	
Total	14	14	0	0	11	3	Mean 122
%		100	0	0	79	21	

d Deep enough, but not in vicinity of vascular bundle.

Compared with *Myzus persicae* and *M. circumflexus*, *Macrosiphum gei* was remarkabl the consistency shown by it in the intracellular method of penetration, and for the percentage of aphides actually feeding in the phloem tissue (Pl. 14, fig. 7). Its perform in these respects is compared with that of the other aphides in Tables 4 and 5.

According to these observations a very high percentage of *M. gei* feed in the vas tissue. This differs from the findings of Dykstra & Whitaker (1938) with *Macrosi* (*Illinoia*) *solanifolii* (probably synonymous with *M. gei*) on potato. These workers sugg that the reason why this aphis was a less efficient vector of leaf roll than *Myzus per*.

M. circumflexus, and *M. solani* was because it was the only one of the four studied which fed less than 50% of the time in the vascular region.

TABLE 4. *Aphides feeding in the phloem after 1–24 hr. Comparison between* Myzus persicae, M. circumflexus, *and* Macrosiphum gei

Aphides	Total no. of aphides	Feeding in phloem	%
Myzus persicae on tobacco	9	5	55
M. persicae on sugar beet	21	13	62
M. circumflexus on tobacco	16	12	75
Macrosiphum gei on tobacco	14	11	79

TABLE 5. *Comparison between the penetration methods of* Myzus persicae, M. circumflexus, *and* Macrosiphum gei *on tobacco, and* Myzus persicae *on sugar beet*

Aphides	Total no. of aphides	% doubtful	% intracellular	% intercellular	Penetration through stoma
M. persicae on tobacco	32	13	59	28	1
M. circumflexus on tobacco	31	23	13	64	3
M. gei on tobacco	14	0	100	0	0
M. persicae on sugar beet	39	10	59	31	2

Smith (1926) stated that *Myzus persicae*, *M. circumflexus*, and *Macrosiphum solanifolii* (*M. gei*) on potato "appear to favour an intercellular method of penetration" though *M. solanifolii* "occasionally follows an intracellular course to the phloem". Table V shows that the behaviour of these three insects on tobacco and of *Myzus persicae* on sugar beet is contrary to the results obtained by Smith on potato. All the aphides, especially *Macrosiphum* spp. preferred the intracellular method of penetration except *Myzus circumflexus* which penetrated by this method in only 13% of the preparations studied.

(4) Myzus persicae *on sugar beet*

Aphides were fixed on sugar-beet leaves after feeding times of 15 min., and 2 and 24 hr. Table 6 shows that only two out of eighteen aphides succeeded in penetrating the phloem after 15 min. feeding. Some of the unsuccessful aphides had reached cells near the phloem, others penetrated the xylem, or had penetrated the leaf sufficiently deeply though not near a vascular bundle (Pl. 14, figs. 8, 9). Even after 2 hr. only 50% of the insects were feeding in the phloem, though here a higher percentage of the unsuccessful ones had reached the edge of the vascular bundle (Pl. 14, figs. 10, 11).

Tables 4 and 5 show that the feeding habits of *M. persicae* on sugar beet and on tobacco are very similar and, like *Macrosiphum gei*, well over 50% of the penetrations are intracellular. The rate of penetration of *Myzus persicae* on sugar beet and its bearing on the activity of the aphides in the transmission of sugar-beet yellows virus will be discussed in another section.

Toxic or other effects on the host plant

Smith (1926) stated that in the case of *Myzus persicae* the effect of the salivary secretions on the host (potato) is considerable and greater than that of *M. circumflexus* and *Macrosiphum solanifolii* (Ashmead). Adjacent to the path of the stylet track the cell contents are disorganized, the chloroplasts and nuclei frequently being destroyed. He also observed that

TABLE 6. *Method of feeding and penetration rates of* Myzus persicae *on sugar beet*

Feeding time	Slide no.	Method of penetration: Intracellular	Method of penetration: Intercellular	Method of penetration: Doubtful	In phloem	Not in phloem	Depth of penetration μ
15 min.	1	.	+	.	.	+*a*	42
	2	+	.	.	.	+	160
	3	.	.	+	.	+	?
	4	.	+	.	+	.	110
	5	.	+	.	.	+*b*	60
	6	.	.	+	+*e*	.	140
	7*a*	+	.	.	.	+*c*	86
	7*b*	+	.	.	.	+	40
	8	+	.	.	.	+	110
	9	.	+	.	.	+*d*	90
	10	+	.	.	.	+	100
	11	.	.	+	.	+	?
	12	.	+	.	.	+*a*	130
	13	+	.	.	.	+	84
	14	.	.	+	.	+	?
	15	+	.	.	.	+	?
	16	+	.	.	.	+	84
	17	+	.	.	.	+*d*	?
Total	18	9	5	4	2	16	Mean 96
%		50	27	23	11	89	
2 hr.	1	.	+	.	+	.	120
	2*a*	.	+	.	.	+*c*	140
	2*b*	+	.	.	+	.	230
	3	+	.	.	+	.	120
	4	+	.	.	+	.	108
	5	+	.	.	+	.	74
	6	+	.	.	+	.	186
	7	.	+	.	.	+*c*	280
	8	+	.	.	.	+	62
	9*a*	+	.	.	.	+*c*	138
	9*b*	+	.	.	.	+	250
	10	+	.	.	.	+	76
Total	12	9	3	0	6	6	Mean 149
%		75	25	0	50	50	
24 hr.	1	+	.	.	+	.	80
	2	.	+	.	+*c*	.	220
	3*a*	+*s*	.	.	+	.	100
	3*b*	+	.	.	+	.	80
	4	.	+	.	.	+*c*	100
	5*a*	.	+	.	+	.	170
	5*b*	.	+	.	+	.	180
	5*c*	+	.	.	+	.	80
	6	+	.	.	.	+	104
Total	9	5	4	0	7	2	Mean 124
%		55	45	0	77	22	
Total	39	23	12	4	15	24	
%		59	31	10			

a, near xylem; *b*, in xylem; *c*, near phloem; *d*, deep enough, but not in vicinity of vascular bundle; *s*, penetration through stoma; *e*, through xylem.

"in stem punctures, as in the leaf, the excess of saliva is so great that the epidermal cells and those underlying are entirely destroyed giving rise to a cup-like depression on the stem surface". Wound reaction in the phloem with abnormal division of the cells could sometimes be seen. Toxic reaction to *Myzus circumflexus* and *Macrosiphum solanifolii* by the cells of the host were similar to *Myzus persicae* though slightly less marked.

In this study of *M. persicae* on tobacco and sugar beet, and *Macrosiphum gei* and *Myzus circumflexus* on tobacco no toxic reaction of the host plant to the insects' saliva was observed. Apart from the safranin staining stylet track there did not appear to be any accumulation of excess saliva in the host plant. The reactions observed above may be due to greater sensitiveness of the potato to the saliva of insects, or to the fact that none of the aphides discussed here were fed for more than 24 hr. on the host plant. Brandes (1923), however, who fixed colonies of *Aphis maidis* feeding on leaves clipped from the host plant, found no apparent wound reaction on the part of the plant visible either in the phloem or from the outside.

Discussion

Investigation of the feeding habits and penetration rates of three aphides *Myzus persicae*, *M. circumflexus* and *Macrosiphum gei* concerned in the transmission of the three strains of *Hyoscyamus* virus III, and of cucumber virus I and potato virus *Y* show that efficiency of the vectors does not depend on localization of these viruses in the vascular tissue of the plant such as Bennett (1934) believes to be the case in curly-top disease of beet. In general, infectivity of the aphides does not seem to be associated with any particular tissue tapped during feeding or with any particular method of penetrating the plant. The one possible exception to this is discussed later.

It has been shown (Watson, 1938; Watson & Roberts, 1939) that "an increase in vector efficiency corresponding with an increase in the times for which the aphides fasted before feeding on the infected plants was shown by all the aphides in their transmission of all the viruses" and that the vector efficiency of all the aphides decreased with increasing feeding time on the infected plants if the aphides had previously been starved.

This decrease in efficiency in the case of *Myzus persicae* with *Hyoscyamus* virus III was as follows: of a total of forty-five plants (five plants per treatment repeated on nine occasions) infection feeding of 2 min. resulted in thirty-four infections; 15 min., eighteen infections; and 4 hr., five infections. Watson (1938) suggested that the most likely explanation for the effect of preliminary fasting and infection feeding times is that some substance is produced by the insect when feeding which inactivates the virus or forms an uninfective compound with it. This seems so far to be the only reasonable explanation for the effect that preliminary fasting and short infection feeding have on the infectivity of the aphides.

If the virus were localized in the superficial tissues of the leaf instead of in the phloem this still could not account for the drop in infectivity between 5 and 15 min. nor could it account for the difference between starved and unstarved aphides. Table 1 shows that in the 5 min. feedings of *M. persicae* on tobacco two out of eleven aphides had almost succeeded in reaching the phloem. The others had reached various points between the vascular bundle and the epidermis. If we suppose that in the short feedings the only insects which were fixed on the leaves were particularly rapid or good feeders, there is still not enough difference between the depth penetrated by these and the 15 min. insects (only one of which penetrated the

phloem) to account for a major difference in infectivity on the grounds that the virus might be more easily available to the aphides in the superficial leaf tissues.

Localization of the virus, or its availability to the insect might, however, account for the difference in behaviour between *M. circumflexus* and *M. persicae* in the transmission of cucumber virus I. *M. circumflexus* shows typical decrease in infectivity between 5 and 15 min. feeding. It also shows a higher degree of relative efficiency with this virus than *M. persicae* whereas with both *Hyoscyamus* virus III and potato virus *Y* it is a less efficient vector than *M. persicae*. When cucumber virus I is transmitted by *M. persicae* there is no decrease in vector efficiency between 5 and 15 min. and on some occasions maximum efficiency is not reached until 15 min. Watson & Roberts (1939) suggested that at the time of optimum infectivity (less than 5 min. for *M. persicae*) the insect has not reached tissues where the virus is most highly concentrated, and that by the time these tissues are reached the virus is already being inactivated by the secretion produced during feeding. Small differences in penetration rates, or partiality of *M. circumflexus* for the virus-infected tissues were advanced as reasons which might enable this aphis to reach these tissues before potential infectivity had been very greatly reduced.

From data presented in Tables 1 and 2 it seems that *M. circumflexus* penetrates more deeply than *M. persicae* during 5 min. feeding, the difference being between 57 μ for *M. circumflexus* and 47 μ (starved and unstarved aphides) or 52 μ for starved *M. persicae* (Pl. 14, figs. 1–4). Too few insects were fixed for any great reliance to be placed on these latter figures but it is possible also that the intercellular method of penetration preferred by *M. circumflexus* is more efficient than the intracellular method of *M. persicae* in reaching the tissues where cucumber virus I is most concentrated.

The problem of the insect transmission of sugar-beet yellows virus, a member of a group which survives in its vector for comparatively long periods, differs from that of viruses such as *Hyoscyamus* virus III, potato virus *Y*, and cucumber virus I which survive in their vectors always for a shorter period than that during which they remain active in untreated infective plant sap. The names "persistent" and "non-persistent" viruses have been proposed to describe these two groups (Watson & Roberts, 1939).

Unpublished experiments on the transmission of sugar-beet yellows virus by *M. persicae* have shown that the efficiency of the vectors increases with increasing feeding time on infected plants. Very rarely could infections be obtained after 7 min. feeding, and only when the feeding time on the healthy plants was more prolonged. The shortest time of total feeding in which an infection was obtained was 30 min. After $\frac{1}{2}$ hr. on the infected and 24 hr. on the healthy plants, 16 % successful infections were obtained. This percentage increased to 66 % for 2 hr. and to 82 % for 24 hr. infection feeding. The number of aphides which penetrate to the phloem, also increases with increasing feeding time, i.e. after 15 min. 11 % of the insects were feeding in the phloem, after 2 hr. 50 %, and after 24 hr. 77 % (Table 6). These rates of increase for successful infections and for the number of insects feeding in the phloem with increasing infection feeding times are not strictly comparable, as, on the one hand, five aphides were used per plant to obtain a successful infection, and on the other, it is probable that the only aphides fixed were those that had deeply penetrated the leaf. The transmission experiments with sugar-beet yellows virus also showed that there was an independent increase in infectivity of the vectors with increasing feeding times on the healthy plants. Bennett (1934), working on the transmission of curly-top disease of sugar

beet by *Eutettix tenellus* found that few insects became infective by feeding on the non-vascular tissue.

Although there is no proof that sugar-beet yellows virus must be obtained from and inoculated into the phloem, some of the results suggest it. Even when fed for 24 hr. or longer all the aphides do not cause infections, and in all the preparations fixed a few aphides consistently failed to feed in the phloem even after 24 hr. Insects rarely reach the phloem in 15 min. and few infections are obtained from insects fed for similarly short times. If phloem feeding were essential, these observations would explain non-transmissions of the virus. Factors, however, such as the amount of virus ingested by the aphides, and possibly the time during which potential infectivity is retained, undoubtedly contribute to the results obtained in the transmission experiments, and could explain them independently of the feeding behaviour of the aphides in the plant.

Summary

1. The feeding habits and penetration rates of three aphides *Myzus persicae*, *M. circumflexus*, and *Macrosiphum gei* on tobacco, and of *Myzus persicae* on sugar beet were investigated in relation to their transmission of *Hyoscyamus* virus III, potato virus *Y*, cucumber virus I, and sugar-beet yellows virus.

2. Neither *M. persicae* or *M. circumflexus* were found to reach the phloem after 5 min. feeding on tobacco. Very few *M. persicae* penetrated the phloem in 15 min. on tobacco or sugar beet. Even after 24 hr. a few aphides do not penetrate the phloem but feed on non-vascular tissue.

3. *Myzus persicae* and *Macrosiphum gei* were found to penetrate by the intracellular method in more than 50% of the slides examined, and *Myzus circumflexus* in less than 50%. *Macrosiphum gei* showed a higher percentage of phloem penetrations in 1–24 hr. than the other aphides.

4. There were no visible toxic effects on the part of the host plants to the insects' saliva with either tobacco or sugar beet.

5. A possible correlation between the behaviour of *Myzus persicae* and *M. circumflexus* with cucumber virus I and localization of this virus in the leaf is discussed.

6. Increased infections with increased feeding times on both infected and healthy plants, in the transmission of sugar-beet yellows virus by its vector *M. persicae* is discussed in relation to phloem feeding.

Thanks are due to Mrs W. G. Cochran (B. I. M. Mitchell, Ph.D.) for permission to use data included in Table 1, and to Mrs M. A. Watson for helpful criticism and advice.

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EXPLANATION OF PLATES 14 AND 15

Plate 14

Fig. 1. Unstarved *M. persicae* after 5 min. on tobacco; penetration through a stoma. Stylets only. ×420.

Fig. 2. Unstarved *M. persicae* after 5 min. on tobacco. Stylets and stylet track. ×420.

Fig. 3. *M. circumflexus*. Depth penetrated after 5 min. on tobacco. Stylets and stylet track. ×420.

Fig. 4. Deep 5 min. intercellular penetration by *M. circumflexus*: (*a*) entry of stylets, (*b*) end of stylet track immediately below centre of picture entering from the right. ×420.

Fig. 5. Intercellular penetration by *M. circumflexus* after 1½ hr. Feeding in phloem. ×420.

Fig. 6. *M. circumflexus* after 24 hr. The stylet track can be seen near the phloem. Stylets curved to xylem. ×420.

Fig. 7. *Macrosiphum gei* after 1 hr. End of stylet track forked. Feeding in phloem. ×420.

Plate 15

Fig. 8. *M. persicae* after 15 min. feeding on sugar beet. Penetration to non-vascular tissue. ×420.

Fig. 9. *M. persicae* after 15 min. feeding on sugar beet. End of stylets in xylem. ×420.

Fig. 10. *M. persicae* on sugar beet. Stylets in phloem of vascular bundle after 2 hr. feeding. ×420.

Fig. 11. *M. persicae* on sugar beet. Stylets in phloem after 2 hr. feeding. ×105.

Fig. 12. *M. persicae* on sugar beet. Stylets have passed through xylem to reach phloem. After 24 hr. feeding. ×105.

(*Received* 12 *February* 1940)

Fig. 1.

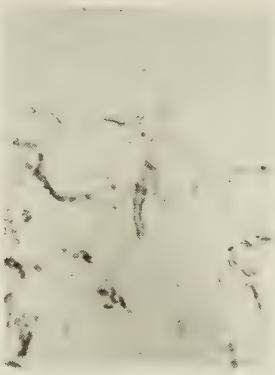


Fig. 2.

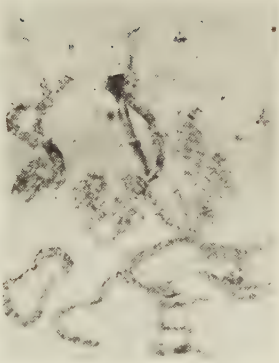


Fig. 3.

Fig. 4*a*.

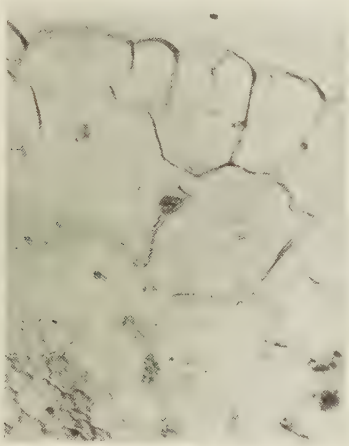


Fig. 4*b*.

Fig. 5.

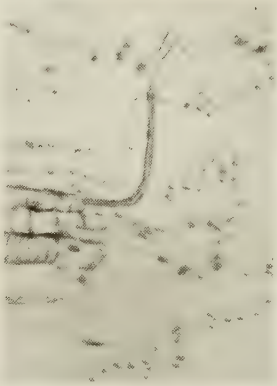


Fig. 6.

Fig. 7.

ROBERTS.—Studies on the Feeding Methods and Penetration Rates of *Myzus persicae* Sulz., *Myzus circumflexus* Buckt., and *Macrosiphum gei* Koch. (pp. 348–358)

Fig. 8.

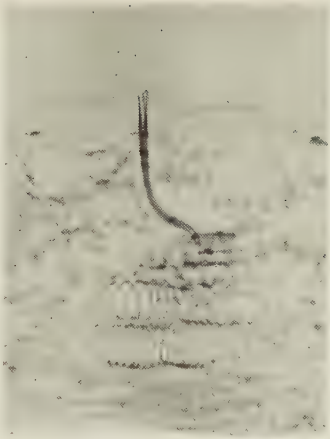


Fig. 9.

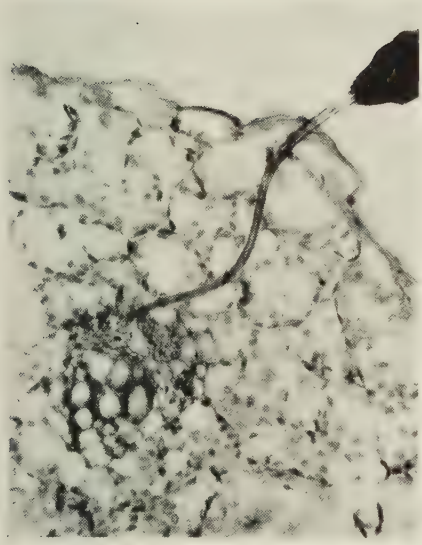


Fig. 10.

Fig. 11.

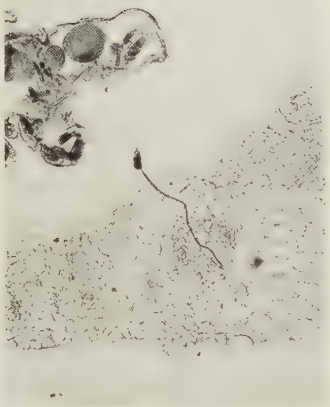


Fig. 12.

ROBERTS.—Studies on the Feeding Methods and Penetration Rates of *Myzus persicae* Sulz., *Myzus circumflexus* Buckt., and *Macrosiphum gei* Koch. (pp. 348–358)

A QUANTITATIVE STUDY OF THE INTERACTION OF VIRUSES IN PLANTS

By T. S. SADASIVAN

Plant Pathology Department, Rothamsted Experimental Station, Harpenden

(With Plate 16 and 1 Text-figure)

ATTEMPTS to infect plants with mixtures of virus strains led to the discovery that plants infected with one strain acquire an immunity, or resistance, to infection with other strains of that virus, although still remaining susceptible to unrelated viruses (Thung, 1931; Salaman, 1933, 1938; Kunkel, 1934; Caldwell, 1935). The mechanism underlying this acquired immunity is unknown, but it is of the non-sterile type, the immunity existing only so long as a plant contains one strain in an active form. Most workers have merely shown that plants systemically infected with avirulent strains fail to develop any further symptoms when reinoculated with virulent strains. In the work described in this paper an attempt has been made to apply quantitative methods to the problem, the actual extent of resistance acquired by infection with one strain being measured by counts of local lesions and not by eye observation of the severity of systemic symptoms

METHODS

So that some idea of the general application of the results could be obtained, the experiments were done with both potato virus "*X*" and tobacco mosaic virus, two unrelated viruses with widely different properties. Two strains of each virus were used, *S* and *G* of potato virus "*X*" (Salaman, 1938) and tobacco mosaic virus and tomato aucuba mosaic virus. "X^S" causes necrotic local lesions and necrotic systemic symptoms in both tobacco (var. White Burley) and *Nicotiana glutinosa*, whereas "X^G" causes only a faint systemic mottle in these plants. Common tobacco mosaic virus causes necrotic local lesions in *N. glutinosa* and systemic mottling symptoms only in *N. sylvestris* and tobacco, whereas aucuba mosaic virus causes necrotic local lesions in all three; aucuba mosaic virus usually becomes systemic in tobacco and rarely does so in *N. sylvestris*.

In this work the experimental designs usually employed for lesion counts, such as the half-leaf method and the Latin square, could not be used, for one-half of the inoculations with either "X^S" or aucuba mosaic virus had to be made to healthy plants and the other to plants already infected. In each experiment, therefore, the treatments were all replicated at least ten times, to reduce differences arising from individual plant variations. As it was also frequently necessary to use leaves of different sizes and ages, the usual method of recording lesions as number per leaf had to be abandoned. Instead they were recorded as the numbers per 100 sq. cm. of inoculated leaf. To obtain the area of the leaves their lengths and breadths were measured and the products of these multiplied by a factor which was found to be constant for each species. The areas of sixty leaves each of tobacco, *N. sylvestris*, and *N. glutinosa* were found by tracing on squared paper, and their lengths and breadths at the widest points measured. In spite of the difference in sizes and ages of the leaves measured, the ratio length times breadth to area was practically constant for each species. That for tobacco varied from 0·67 to 0·73 (average 0·7), for *N. sylvestris* from 0·72 to 0·76 (average 0·74), and for *N. glutinosa* from 0·87 to 0·89 (average 0·88).

Inoculations were made by rubbing whole leaf surfaces as evenly as possible with the forefinger dipped in the experimental fluids. Infective preparations of three kinds were used: (1) crude saps, (2) clarified saps prepared by heating to 60° C., and centrifuging for 10 min. at 3000 r.p.m., and (3) purified virus preparations made by the methods described by Bawden & Pirie (1937).

Experimental

In preliminary experiments the whole leaf surfaces of plants were rubbed with either water, or crude sap from plants infected with tobacco mosaic virus, potato virus "*Y*", or potato virus "X^G". At daily intervals these leaves were rubbed over their whole surfaces with crude aucuba mosaic sap diluted 1/100 with water. The average number of lesions per 100 sq. cm. produced on these plants is given in Table 1. It will be seen that the only plants to become resistant to aucuba mosaic virus were those that had previously been inoculated with tobacco mosaic virus. These show a steady decrease in susceptibility to aucuba mosaic virus; with increasing time they were infected with tobacco mosaic virus up to 6 days when they were quite immune. The aucuba mosaic lesions on plants infected with "X^G" or virus "*Y*" were similar to those on the healthy controls, large circular necrotic spots, whereas, on those infected with tobacco mosaic virus, in addition to being fewer, the lesions were smaller and had much more uneven edges. In similar experiments in which rubbed plants were reinoculated at intervals with "X^S", the only plants to show a reduction in susceptibility were those previously rubbed with "X^G". These tests sufficed to show that the acquired immunity is specific, being restricted to strains of one virus.

Although the presence of an unrelated virus in a plant had no effect in the number or type of lesion produced by aucuba mosaic virus or "X^S", unrelated viruses may have interactions inside a plant; e.g. tobacco mosaic virus and potato virus "*X*" together cause acute necrotic diseases in tobacco and tomato that neither can cause alone. Similarly, in these local lesion tests, it has been found that plants infected with tobacco mosaic virus show necrotic, spot-like local lesions when reinoculated with "X^G" and vice versa, although neither virus alone produces visible local lesions on this plant.

Table 1. *Effect of previous inoculation of plants on number of local lesions produced by aucuba mosaic virus*

Days between inoculations	No. of lesions per 100 sq.cm. on plants previously rubbed with			
	Water	Virus "*Y*"	Virus "X^G"	Tobacco mosaic
1	675	762	639	220
2	715	675	577	185
3	765	650	578	80
4	718	602	675	70
5	635	627	716	10
6	618	687	710	0
7	575	615	680	0
8	645	610	600	0
9	712	627	575	0
10	676	595	585	0

These results could be explained on two hypotheses: (1) that related viruses have a mutually destructive effect or (2) that in the plant there is an intense competition between them. To test the first possibility a number of *in vitro* tests were made. Crude "X^S" sap was mixed in different proportions with sap from plants infected with "X^G" and with sap from healthy tobacco plants and from those infected with various viruses, and these mixtures were tested for their infectivity. Tests were also made with aucuba mosaic virus diluted similarly. The results of one such experiment are given in Table 2.

TABLE 2. *Effect on infectivity of "X^S" and aucuba mosaic virus of diluting in different fluids*

Preparation diluted	Diluent	No. of lesions at dilution of 1/20	1/100	1/500	1/2500	1/12,500
Crude "X^S"	Tobacco sap	196	54	20	4	1
	Crude virus "Y" sap	180	85	37	8	2
	Crude mosaic virus	207	64	34	6	1
	Crude "X^G" sap	30	9	0	0	0
	Water	361	183	62	7	4
Aucuba mosaic	Tobacco sap	134	86	32	23	8
	Crude "X^G" sap	131	78	21	11	2
	Crude mosaic virus	43	15	4	0	0
	Water	383	346	180	58	18

Several similar experiments have been done with the same results, saps from healthy and infected plants inhibiting the infectivity of both "X^S" and aucuba mosaic virus. The sap from plants infected with unrelated viruses has no significantly different effect from healthy plant sap, but that containing related viruses has a much greater inhibitory action. In addition to those given in Table 2, "X^S" and aucuba mosaic virus were mixed with sap expressed from plants infected with the following viruses: *Hyoscyamus* 3, tobacco etch, tobacco ringspot, and cucumber virus 1. All had the same inhibitory effect as healthy plant sap. On the other hand when "X^S" was mixed with sap from plants infected with "X^L", greater inhibition was obtained, comparable with that produced by "X^G". The materials in healthy and infective saps responsible for the non-specific reduction in infectivity are not removed by centrifugation at 3000 r.p.m. after heating to 60° C., for in experiments comparing the inhibitory effects of crude and clarified saps no significant differences were obtained. The greater specific reduction in infectivity caused by diluting with sap containing related viruses seems to be due to the presence of the viruses themselves and not to any other specific product of virus activity, for a similar reduction in the infectivity of purified aucuba mosaic virus occurs when it is diluted with purified tobacco mosaic virus free from all detectable impurities. Similarly, when aucuba mosaic virus is mixed with crude boiled sap of tobacco mosaic virus, the lesion production of aucuba mosaic is almost identical with that produced when mixed with healthy sap of tobaccos (Pl. 16, fig. 1).

At first sight the results of these dilution experiments suggest that related viruses may have a mutually inactivating effect *in vitro*. This is especially so as the reduction in the number of lesions produced by crude aucuba mosaic sap diluted 1/100 with tobacco mosaic sap is greater than that when plants are first inoculated with the tobacco mosaic virus and then reinoculated with aucuba mosaic virus diluted 1/100 with water. However, the results of other experiments show that any interactions between related viruses occur within the plant and not *in vitro*. One of the two effects *in vitro* might cause loss of infectivity; tobacco mosaic might actually destroy aucuba mosaic virus or it might act merely as an inhibitor of infectivity such as trypsin and some other proteins do. If the first were true incubating the mixtures would be expected to give increased reduction of lesions, for such inactivation would not be expected to occur immediately. However, no significant differences were found between the number of aucuba lesions when the mixtures were rubbed on to tobacco or *N. sylvestris* immediately and when they were incubated for some hours. If tobacco mosaic virus merely acts as an inhibitor, its effects would be expected to be determined by

the total amount of protein present, whereas it is not. The infectivity of tobacco mosaic virus can be destroyed with formaldehyde (Stanley, 1934) without denaturing the protein or altering most of its physical and chemical properties. Mixtures of purified aucuba mosa virus were made with the same amount of purified tobacco mosaic virus which in some w fully active and in others inactivated by varying amounts with formaldehyde. The reducti in lesions when these mixtures were inoculated to tobacco and *N. sylvestris* was alwa proportional to the amount of infective tobacco mosaic virus. If either of these *in vit* reactions occurred loss of infectivity would be expected on every host plant, but this is n so. The reduction only happens on host plants in which aucuba mosaic produces local lesio and tobacco mosaic virus becomes systemic. When dilute mixtures of the two viruses we inoculated to *N. glutinosa*, in which both give only local lesions, there is no evidence inhibition of the local lesion production of either of the viruses, for the number of lesio produced are what would be expected from the total amount of virus present in the mixtur

The results so far examined show that virus strains producing systemic symptoms redu the number of lesions caused by other strains when mixed *in vitro*. This suggests that a interaction occurs within the plant. Further experiments with tobacco mosaic, aucu mosaic, potato virus "X^S" and "X^G" *in vivo* have lent support to this view and suggest th in the plant tissues there is an intense competition between related viruses, and that t resistance acquired to one depends on the amount of the other already present. Experimer were performed where different parts of leaves of tobacco were inoculated with tobac mosaic and "X^G" viruses and such areas reinoculated with aucuba mosaic and "X viruses respectively. In one of these experiments *tips* of leaves were inoculated with eith "X^G" sap or tobacco mosaic sap and then reinoculated with "X^S" or aucuba mosaic vir at daily intervals. The "X^G" areas were quite immune to "X^S" after 12 days and the tobac mosaic areas immune to aucuba mosaic virus in 4 days. The *bases* of such leaves showed incomplete inhibition even after 20 and 24 days. When *bases* of leaves were similarly in culated, essentially the same type of inhibition was obtained in the "X^G" and tobacco mos inoculated areas, but the *tips* of such leaves acquired resistance to "X^S" and aucuba mos much more slowly and incompletely. The results of this experiment are given in Table

In addition to the leaves reinoculated with aucuba mosaic virus some of the *N. sylvest* plants inoculated in *tips* and *bases* with tobacco mosaic virus were sampled at different perio of incubation by cross inoculation on to *N. glutinosa* for their virus content. Inoculat areas, whether *tips* or *bases*, soon contained a high concentration of the virus, whereas vir entered and increased slowly in uninoculated areas. When *tips* of leaves were initia inoculated, the virus moved down to the *bases* and reached a high concentration where with the leaves where *bases* were inoculated, the *tips* rarely obtained such a high concent tion. This once again corroborates Samuel's (1934) suggestion that the movement of viru is related to the translocation stream in the plant and that therefore movement from *tips* *bases* is more efficient than *bases* to *tips*. Although this experiment could not be repea for "X^G" virus, which does not produce visible local lesions, it probably moves and mu plies in a similar way though at a lower level than tobacco mosaic virus.

Thus, it appears from the above experiments and the data presented in Table 3 t "X^G" and tobacco mosaic virus bring about rapid inhibition to "X^S" and aucuba mos virus in the initially inoculated areas, but further inhibition in other parts of the pl depends on the efficient spread of "X^G" and tobacco mosaic virus from the inoculated areas

TABLE 3. *Comparison between "X^S" and aucuba mosaic lesions produced in tips and bases of leaves inoculated with "X^G" and tobacco mosaic viruses in* N. Tabacum *and* N. sylvestris *respectively*

Period of incubation days	No. of lesions per 100 sq. cm. leaf area							
	Tips alone inoculated with				Bases alone inoculated with			
	"X^G"		T.M.V.		"X^G"		T.M.V.	
	Tips	Bases	Tips	Bases	Bases	Tips	Bases	Tips
1	723	829	163	405	380	623	101	650
2	583	540	76	371	451	664	118	489
3	331	612	32	226	253	729	34	260
4	406	568	8	402	190	603	14	222
5	18	411	0	385	26	977	0	231
6	8	462	0	365	4	656	0	208
8	16	145	0	112	9	600	0	138
10	8	87	0	317	3	262	0	109
12	5	110	3	48	12	212	0	53
14	0	195	6	75	0	177	5	26
16	0	155	8	13	0	215	9	49
20	0	18	13	12	0	265	8	50
22	—	—	3	8	—	—	10	38
24	—	—	3	9	—	—	9	42

into the adjoining leaf tissues, and their multiplication therein. In other words the higher the concentration of the two strains the greater the inhibition to "X^S" and aucuba mosaic virus. This process of natural movement within the host, however, does not seem to bring about as complete or as rapid an inhibition as direct inoculation of areas. The results suggest that the direction of movement of viruses is dependent on the place of inoculation, for it has been shown that movement from *tips* to *bases* of leaves is comparatively more efficient than from *bases* to *tips* as also evidenced by inhibition tests of "X^S" and aucuba mosaic viruses. Since all these observations were made on parts of leaves of one age, further experiments were conducted with tobacco mosaic and aucuba mosaic viruses on young, medium and old leaves of *N. sylvestris*. Inoculations were made as follows: three sets of plants were inoculated with tobacco mosaic virus on one leaf each of three ages, young, medium and old. These plants were reinoculated at 2-day intervals up to 20 days with aucuba mosaic virus on the initially inoculated leaves as well as on the other uninoculated leaves of three ages. The results, Table 4, show that tobacco mosaic inoculated areas in all three ages completely resist aucuba mosaic lesions at the end of 12 days. In contrast young leaves that were not initially inoculated with tobacco mosaic virus although resistant are not immune even after 20 days, though tobacco mosaic systemic symptoms appear after 10 days' incubation. Such incomplete inhibition, however, is greater in uninoculated medium-aged leaves and more so in old leaves.

In experiments where three ages of leaves from *N. sylvestris* plants were infected with tobacco mosaic virus and were tested on to *N. glutinosa* at different periods of incubation it was found that whatever the age of the leaf receiving the initial inoculation the young leaves always have a higher concentration of the virus than medium and old leaves that become systemically infected. This result contrasts with that obtained when leaves are inoculated and not infected by systemic spread, for then the virus multiplies more rapidly and reaches a higher concentration in medium and old leaves than in young. Indeed, the concentration

of virus in directly inoculated old leaves is almost twice that in the young ones. Correspondingly, the data in Table 4 show that medium and old leaves inoculated with tobacco mosaic virus show a large reduction in the aucuba mosaic lesions in 2 days, whereas similarly treated young leaves take as long as 4 days to bring about the same reduction. One such comparison between the concentration of tobacco mosaic virus in inoculated leaves of *N. sylvestris*, as shown by tests on *N. glutinosa*, and their susceptibility to aucuba mosaic virus is presented in Text-fig. 1. It will be seen that as the concentration of tobacco mosaic virus increases with increased incubation, the number of aucuba mosaic lesions steadily decreases, i.e. the rise of concentration of tobacco mosaic brings about a corresponding rise in the degree of resistance to aucuba mosaic virus.

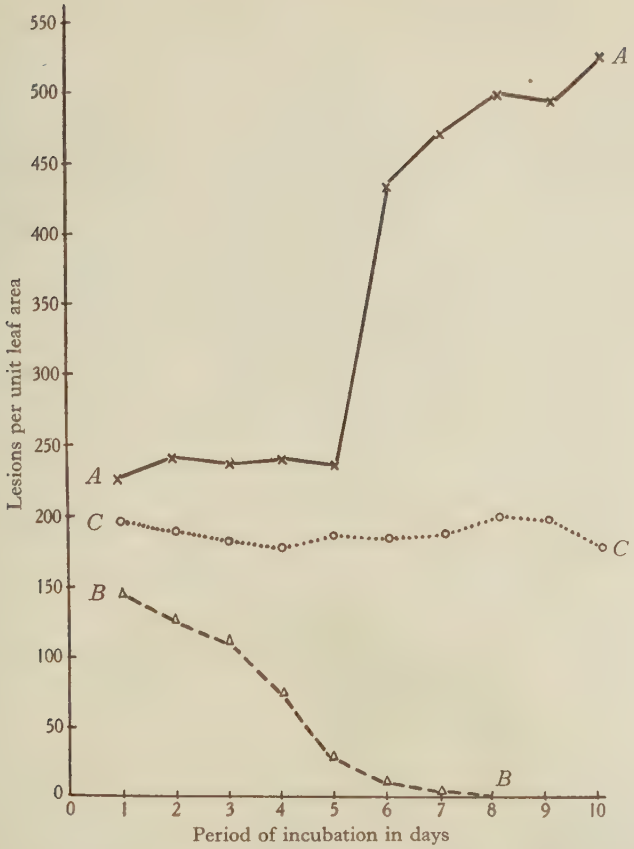
TABLE 4. *Comparison of aucuba mosaic lesions between leaves of three ages where tobacco mosaic virus has been inoculated and also on leaves of the three ages when tobacco mosaic systemic symptoms have appeared*

Period of incubation days	Young leaves directly inoculated with T.M.V.	Young leaves showing T.M.V. systemic symptoms	Medium leaves directly inoculated with T.M.V.	Medium leaves showing T.M.V. systemic symptoms	Old leaves directly inoculated with T.M.V.	Old leaves showing T.M.V. systemic symptoms
2	227	196	350	826	346	1830
4	205	145	337	1166	272	1330
6	187	133	167	979	296	1450
8	100	75	134	484	127	1418
10	23	67	113	584	25	1612
12	8	63	50	557	0	1716
14	0	50	0	603	0	961
16	0	69	0	613	0	824
18	0	79	0	392	0	875
20	0	15	0	253	0	818

Although old and medium leaves, when directly inoculated with tobacco mosaic virus, become resistant to aucuba mosaic virus more rapidly than young ones, the reverse is true when infection occurs as a result of systemic spreading of tobacco mosaic virus. This again is correlated with virus content, for although tobacco mosaic virus multiplies more rapidly in old and medium leaves when they are directly inoculated, when systemic infection occurs multiplication is more rapid and the highest virus content is found in young leaves.

As these observations suggest that the inhibition of aucuba mosaic virus lesions is directly proportional to the concentration of the tobacco mosaic virus in the leaf tissue, regardless of the age of the leaf, it seemed that any method of increasing virus concentration should increase acquired immunity. It is known that carborundum powder incorporated in the inoculum increases the number of local lesions. This increase is believed to result from an abrasive action increasing the number of entry points. A comparison was, therefore, made between sets of *N. Tabacum* and *N. sylvestris* leaves inoculated with crude tobacco mosaic virus sap and another sap to which was added carborundum powder.

These inoculations were made in the centres of laminae and aucuba mosaic virus was reinoculated all over the surface of the leaves at different periods of incubation. The results presented in Table 5 show that a more efficient and earlier inhibition of aucuba mosaic virus could be obtained when carborundum is in the tobacco mosaic virus inoculum than when it is not (Pl. 16, fig. 2).



Text-fig. 1. Correlation between virus content and acquired immunity: curve *A*, tobacco mosaic virus content of *N. sylvestris* as shown by subinoculating to *N. glutinosa*; curve *B*, aucuba mosaic lesions on tobacco mosaic infected *N. sylvestris*; curve *C*, aucuba mosaic lesions on water-rubbed control of *N. sylvestris*.

TABLE 5. *Comparison between aucuba mosaic lesions produced in tobacco mosaic inoculated laminae with and without carborundum powder*

Period of incubation of T.M.V. days	No. of lesions per 100 sq. cm. leaf area											
	T.M.V. + carborundum						T.M.V. alone					
	N. Tabacum			*N. sylvestris*			*N. Tabacum*			*N. sylvestris*		
	Tips	Centres*	Bases	Tips	Centres*	Bases	Tips	Centres*	Bases	Tips	Centres*	Bases
1	1590	321	1285	432	233	200	1470	310	1060	462	296	368
2	1060	60	513	626	283	303	1154	100	977	687	205	400
4	878	0	805	194	15	312	975	35	1052	436	36	457
6	979	0	741	172	16	141	867	8	847	486	32	367
8	825	0	155	160	0	23	713	0	390	240	13	65
10	430	0	15	175	0	65	452	0	140	218	0	16
15	120	0	4	20	0	0	148	0	12	34	0	10

* Areas inoculated with tobacco mosaic virus.

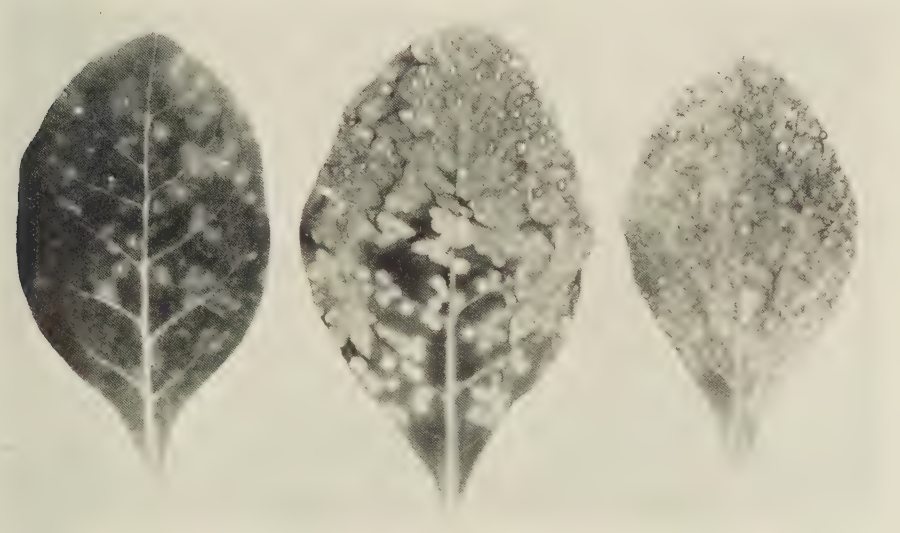
The response to the inhibition of aucuba mosaic lesions in the tobacco mosaic inoculated areas in both types of inoculation is quicker and more efficient in tobaccos than in *N. sylvestris*. Since aucuba mosaic virus produces more lesions in tobaccos than in *N. sylvestris* it could, perhaps, be assumed that *N. Tabacum* is more susceptible than *N. sylvestris* both to aucuba mosaic as well as tobacco mosaic viruses, although tobacco mosaic virus does not produce visible necrotic lesions in either of the two hosts. On this assumption the number of tobacco mosaic virus infective units entering tobacco leaves would be greater than those entering *N. sylvestris* leaves. Thus it seems that *N. Tabacum* acquires resistance to aucuba mosaic virus when vaccinated with tobacco mosaic virus more rapidly than *N. sylvestris* because it is more susceptible to both the viruses.

The only evidence suggesting that the immunity is not merely a result of competition between related virus strains in the plant, is that presented by experiments on mixing viruses *in vitro*. Table 1 shows that mixing of either aucuba mosaic virus with tobacco mosaic or "X^S" with "X^G" viruses produces a specific reduction in the lesions of the former viruses approximately 10 times that produced when mixed with healthy saps. Such a great reduction, however, does not happen when plants inoculated with tobacco mosaic virus or "X^G" are reinoculated with aucuba mosaic virus or "X^S" after 1 day. This apparent anomaly may result from a second type of competition that would occur only when the two strains were inoculated together, i.e. a competition for entry points. In experiments where two viruses were mixed, the concentration of tobacco mosaic virus and "X^G" used for the *in vitro* mixture has always been greater than either aucuba mosaic or "X^S" viruses. A competition for the fixed number of entry points follows, and since the higher number of tobacco mosaic virus and "X^G" infection units utilize the majority of the entry points, only a few are made available for aucuba mosaic and "X^S" virus units. It is highly probable that when tobacco mosaic virus is incubated in the leaves for 1 day, one set of entry points is occupied by the virus, and when such leaves are reinoculated with aucuba mosaic, a fresh number of entry points is made available in the second abrasive process of reinoculation.

Experimental evidence so far examined shows that there is a competition between related strains of viruses. Whether this competition is for a limited amount of material from which viruses reproduce or for the limited parts of a cell wherein the multiplication occurs there is no evidence to suggest. However, summing up the data, *acquired immunity seems to depend on the presence of virus units in a fully active state in cell tissues where they prevent the multiplication of another strain of that virus. This process is highly specific, being confined to the related groups only, and the efficiency merely rests on the concentration and uniform distribution of the virus units, either under natural movement within the plants themselves, or, by direct introduction of active virus units into the cell tissues by mechanical rubbing. The degree of immunity is directly proportional to the number of active units of the protecting strain entering the host cells.*

Summary

When the saps of healthy plants are mixed with potato virus "X^S" or aucuba mosaic virus *in vitro* there is an inhibition of the lesion production on *N. Tabacum* and *N. sylvestris* leaves. Saps containing unrelated viruses also reduce the infectivity to the same extent as healthy saps. However, saps containing strains of related virus, have a greater and specific inhibitory action. Experiments were performed to show that this specific reduction produced



(*a*) (*b*) (*c*)

Fig. 1.

(*a*) (*b*) (*c*)

Fig. 2.

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by related strains of viruses is due to the viruses themselves and not to other metabolic products present in the saps.

Further study by inoculating related strains of viruses *in vivo* has shown that the local lesions of one strain are inhibited by another when the latter systemically infects hosts, and that the efficiency depends on the concentration of the systemically infecting viruses in such hosts. The degree of inhibition of aucuba mosaic virus is directly proportional to the number of active units of tobacco mosaic virus present in the leaf tissues at the time of reinoculation.

I wish to express my indebtedness to Dr J. Henderson Smith and Mr F. C. Bawden for suggesting this problem. Their generous help and criticisms during the various stages of this piece of work are gratefully appreciated.

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EXPLANATION OF PLATE 16

Fig. 1. Lesion on *N. Tabacum* leaves inoculated with aucuba mosaic virus sap mixed *in vitro* as follows: (*a*) with concentrated active tobacco mosaic sap; (*b*) with boiled tobacco mosaic sap; and (*c*) with healthy sap of tobacco.

Fig. 2. *N. sylvestris* half leaves of three ages initially inoculated with tobacco mosaic virus, with and without carborundum powder. Lesions are of aucuba mosaic virus reinoculated after incubation of 1 day. Initial inoculations were: leaf (*a*) left, tobacco mosaic virus alone and right, tobacco mosaic virus + carborundum powder; leaf (*b*) left, tobacco mosaic virus alone and right, water-rubbed control; leaf (*c*) left, water + carborundum-rubbed control and right, tobacco mosaic virus + carborundum powder.

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A STUDY OF THE ZINC-POLLUTED RIVER YSTWYTH IN NORTH CARDIGANSHIRE, WALES

By J. R. ERICHSEN JONES, Ph.D.

Department of Zoology, University College of Wales, Aberystwyth

(With 4 Text-figures)

The river Ystwyth, one of the main rivers of north Cardiganshire (Rheidol, Ystwyth, Aeron and Teifi), has a long history of pollution originating in metalliferous mines. Other rivers in west Wales, notably the Dovey, Teifi and Rheidol, have suffered pollution of a similar nature in the past but have now almost or completely recovered and are protected fisheries. The Ystwyth, despite the fact that the mining industry in its basin has been derelict for nearly forty years, has not recovered and remains the most barren of the rivers running into Cardigan

Fig. 1. General map of the river Ystwyth and the upper reaches of the river Teifi. Disused mine workings which are sources of pollution are indicated by large crossed squares. Other disused mine workings are indicated by small crosses. The 1500-ft. contour is drawn and land over 1500 ft. is shaded. Fauna samples were taken at A, B, C and E. Water samples for analysis were taken at A, B, C, D and F.

Bay; it is completely devoid of fish, almost devoid of vegetation, and has a limited fauna composed almost entirely of insects. This study was undertaken to ascertain the exact nature and extent of the pollution, its sources, and the extent to which the fauna of the river is limited by the chemical condition of its waters and by the peculiar physical characters of the stream which appear to be a heritage of the mining activities of the nineteenth century.

The history of the river. Previous surveys

Of the river's condition in the nineteenth century we have no exact knowledge, but considerable general information is to be found in the *Fifth Report of the Commissioners on River Pollution* (1874). From oral and written evidence gathered by the Commission it appears that early in the nineteenth century the river Ystwyth was a fine clear stream abounding in salmon, sewin and trout, that the cattle grazed on its banks thrived in a remarkable manner, and that the meadows in its valleys had the luxuriant appearance proper to the rich alluvial soil. After 1820 much activity developed at the mines in the Ystwyth valley and fine-grinding machinery was introduced whereby the ores were crushed into a fine state of division before being washed.[1] Supplies of water for ore-washing were obtained by weiring the river and diverting part of its flow into aqueducts. The washing water eventually returned to the stream-bed generally without passing through settling ponds and laden with suspended matter, and vast quantities of broken rock and "skimpings" were cast into the river or heaped upon its banks to be carried away at flood-time. These developments resulted in serious pollution: the river became extensively silted up, all fish disappeared, and the vegetation on land flooded by its waters was damaged or destroyed. The destruction of vegetation rendered the banks more susceptible to erosion and the polluted water and fouled pasture proved poisonous to domestic animals, particularly horses. These evil effects appear to have been attributed to the finely divided ore carried in suspension by the river water.

The Ystwyth valley mines yielded lead, zinc and silver ores. The silver was rapidly exhausted and in the latter half of the nineteenth century the industry declined as one by one the mine workings were abandoned. In 1893 the Cwm Ystwyth mine, one of the largest in the valley and one yielding lead and zinc ores, was closed, and in 1903 the industry became derelict with the closure of the extensive workings at Frongoch. Whether any improvement in the river's condition took place as a result of the closing down of the mines is not recorded. After 1914 there was a redevelopment of activity at the Cwm Ystwyth and Frongoch mines, though it is stated that this did not go beyond the extraction of lead and zinc ore present in the mine-dumps. This revival was short lived and ceased in 1921 since which time no mines have been worked in the Ystwyth valley.

In 1919–21 Carpenter (1924) made a brief survey of the lower Ystwyth and records that at this time the fauna of the river was extremely scanty and limited to nine species, all of these belonging to the animal groups Insecta, Hydracarina and Crustacea-Entomostraca. Analyses of the water of the lower Ystwyth made during 1–15 March 1921 showed that it carried 0·4 mg./l. of lead in solution, but the presence of zinc was not suspected and no attempt was made to detect or estimate this metal. In 1922, after the cessation of mining activity, some improvement in the river's condition became apparent and Carpenter (1924) records the appearance of planarians and many new insect species bringing the total number of species to 26, while two filtered samples of the river water taken on 24 October and 17 November 1922 gave no indication of lead (Carpenter, 1924). As previously, no tests for zinc were applied. The animal groups Vertebrata, Oligochaeta, Crustacea-Malacostraca, Hirudinea and Mollusca remained completely unrepresented.

[1] An account of the methods employed for the washing of lead and zinc ores is given in the *Fifth Report of the Commissioners on River Pollution* (1874, I, 11–12).

In 1924 the river suffered fresh pollution from a new source. The Esgair Mwyn mine (lead and zinc ores) situated in the upper valley of the river Teifi was reopened and in order to avoid pollution of the Teifi a long leet was constructed whereby the mine effluents were conducted into the Ystwyth. This pollution does not appear to have been of a serious order and in 1928 the leet broke down and was not repaired. The Esgair Mwyn mine ceased working shortly afterwards.

Subsequent to Carpenter's surveys of 1919–22 no study was made of the river's chemical and biological condition until the present survey was begun in July 1939. It appears to have been generally recognized that the river showed little sign of recovery, and popular opinion appears to have attributed its barren condition to lead pollution originating in the numerous exposed mine dumps in the valley. On 23 July 1930 the Pollution Committee of the Cardiganshire County Council passed a resolution: "That the River Ystwyth be used for the dumping of effluents, so far as it is practicable to do so, and so save the River Teifi and others from further lead pollution." This resolution, however, was never put into effect as no new mining operations were undertaken.

The present survey

(*a*) *Topography* (Fig. 1)

The river has its source on the Plynlimmon Plateau at an altitude of about 1700 ft., and near the sources of the rivers Wye and Severn. Here the country is wild and barren and the mean annual rainfall is 60–80 in. For about 4 miles the stream flows almost due south, and then pursues a generally westward course to the sea at Aberystwyth on Cardigan Bay. About 8 miles from its source the river passes the highest of the mines in its valley—the Cwm Ystwyth mine; here the valley is very narrow, and on either side of the river precipitous, barren hills rise to nearly 2000 ft. At A, a region selected as a fauna-sampling station representative of the upper course of the river, the stream is 20–30 ft. wide and flows very swiftly between steep, rocky banks; its bed is largely composed of solid rock, and covered with large smooth stones and boulders.

Below the village of Pontrhydygroes, the centre of the mining activity of past years, the river is somewhat inaccessible, having cut a deep gorge about a mile in length. Below its exit from the gorge the river has silted up extensively, its bed is 300–400 ft. wide, occupies the whole of the valley floor, and presents a broad, flat and monotonous expanse of weathered pebbles and rubble over which the stream, at normal level, wanders in an erratic fashion, its course changing slightly from time to time. Here and there are small quiet pools where small quantities of such debris as dead leaves, twigs and mine-dump gravel accumulate, but for the most part the river remains shallow and swift. In this region a second sampling station (B) was selected, representative of the river's middle course.

At Llanafan the valley broadens out suddenly and the Ystwyth pursues its way through cultivated land. At many points along its lower course the river is as extensively silted up as at station B and some impression of its nature may be gathered from Fig. 2, a large scale map of a portion of the river at the village of Llanilar, 6 miles from Aberystwyth. Below Llanilar it is confined to reasonable limits by high banks apparently erected by riparian owners to prevent the flooding of pastures. At fauna-sampling station C, the river is about 70 ft. wide and under the road bridge a deep pool is formed carpeted with gravel, dead leaves and refuse. Immediately above this point the river is shallow and its bed is evenly covered with large pebbles.

From its source to the sea the river measures 29 miles. It receives few tributaries and of these the longest does not exceed 5 miles while some are short impermanent trickles. It is very variable in volume and after heavy rain rises in flood with great rapidity, subsiding with corresponding rapidity on the return of dry weather. When in flood the river is turbid with suspended matter, but normally its waters are exceptionally clear and the suspended matter carried is negligible. The weirs erected near the mine workings have all broken down completely and below the gorge there are no obstacles to the passage of fish.

(*b*) *The sources of pollution* (Figs. 1, 3 and 4)

Along the course of the river and its tributaries are eighteen disused mine workings, the positions of which are indicated in Fig. 1. The majority of these are old levels and shafts from which there is no drainage and which do not constitute pollution sources. The primary source of pollution is the Cwm Ystwyth mine, where an immense dump of mine refuse, consisting mainly of broken rock,

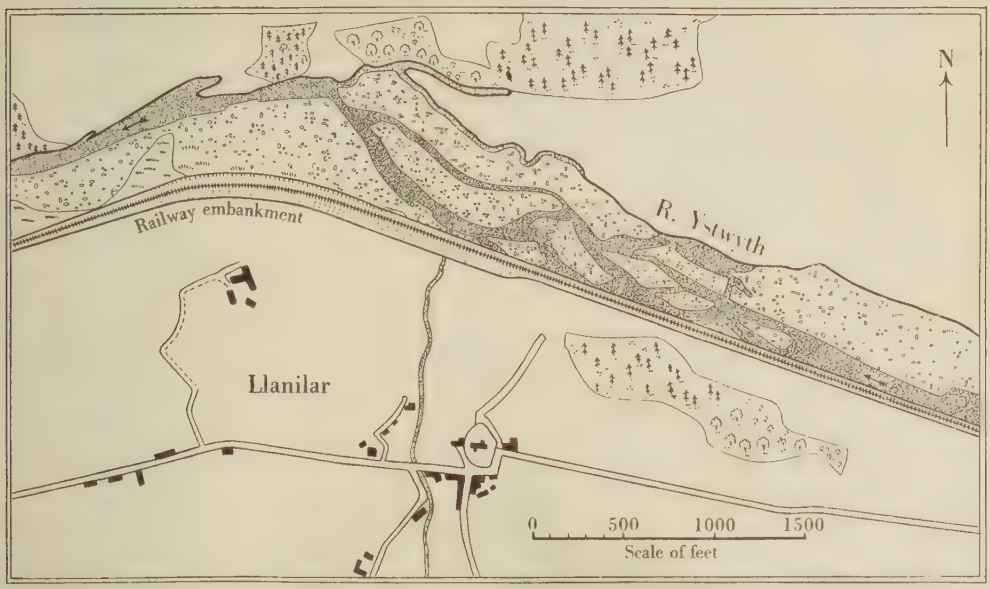

Fig. 2. Large scale map of a portion of the river Ystwyth at Llanilar showing the great width of the river bed and the way the river has silted up with the vast quantities of stones, rubble and gravel that have been carried down from the mine workings. Standard Ordnance symbols throughout.

occupies much of the right side of the valley. Five tributary streams (Fig. 3 *c*, *e*, *f*, *g* and *h*) descend the hillside and cascade over the dump, but they appear to pick up little soluble matter and only streamlets *c* and *f* are at all seriously polluted. At the south-west corner of the dump two streamlets (*a* and *b*) emerge from old adits running beneath the road and run into the river; both are heavily polluted with zinc, and one (*a*) is heavily polluted with lead. Both metals are present in solution, probably as sulphate. These streamlets are stated to be the overflow of water accumulating by percolation in disused shafts. Near the middle of the dump a small trickle of water makes its appearance (*d*), whose exact origin is uncertain; it may be another adit effluent, or it may be a seepage from the dump. This is also heavily polluted with zinc, but contains only a trace of lead.

The second source of pollution is the Frongoch mine, near Pontrhydygroes (Figs. 1, 4). Here the mine dump is flat and no streams drain its surface. On the east side of the dump a swift stream, 3–4 ft. wide, emerges from a low archway and runs down to join the Ystwyth below Pontrhydygroes. The Frongoch stream appears to be an outflow of water from the disused mine workings, is heavily polluted with zinc and slightly polluted with lead. The surface of the dump of the adjoining Wemyss mine is drained by a tributary (Nant Cwm Newydion) which runs to join the Ystwyth 3 miles above Llanilar. This stream appears to pick up some soluble lead and zinc salts from the dump, but is much less heavily polluted than the Frongoch stream. At the Wemyss mine there are a number of open adits, but no water appears to drain from them.

A number of estimations have been made of the *p*H, lead content and zinc content of these various polluting streams and effluents and representative figures are given in Table 1. Zinc was estimated gravimetrically as zinc-mercury thiocyanate and lead colorimetrically as lead sulphide by the methods described by James (1928). All the polluting effluents and streams normally contain a minimum of suspended matter.

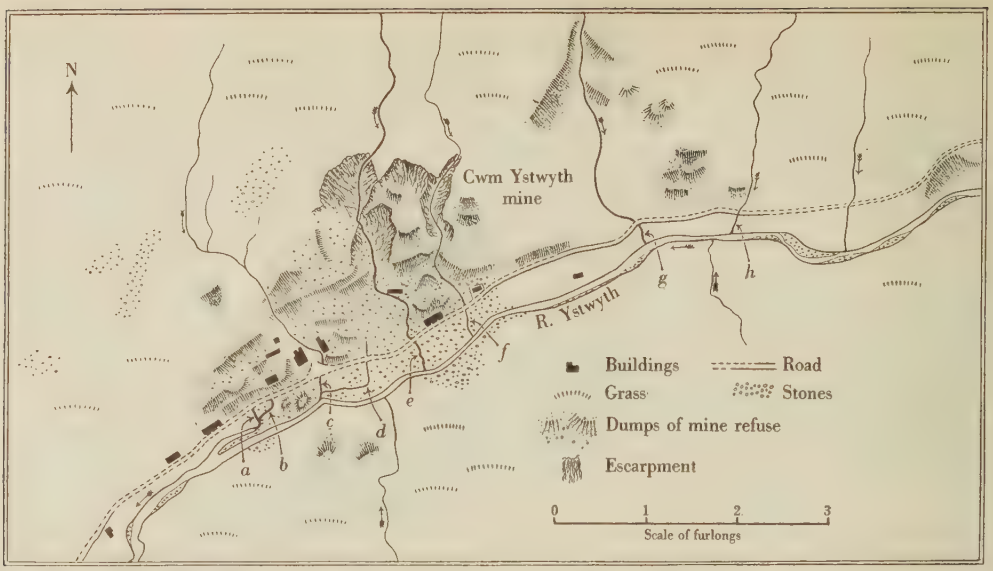

Fig. 3. The Cwm Ystwyth mine, showing the streams draining over the mine dump and the adit effluents running into the river. Samples of water were taken at the points indicated by the arrows. Analytical data for these samples are given in Table 1.

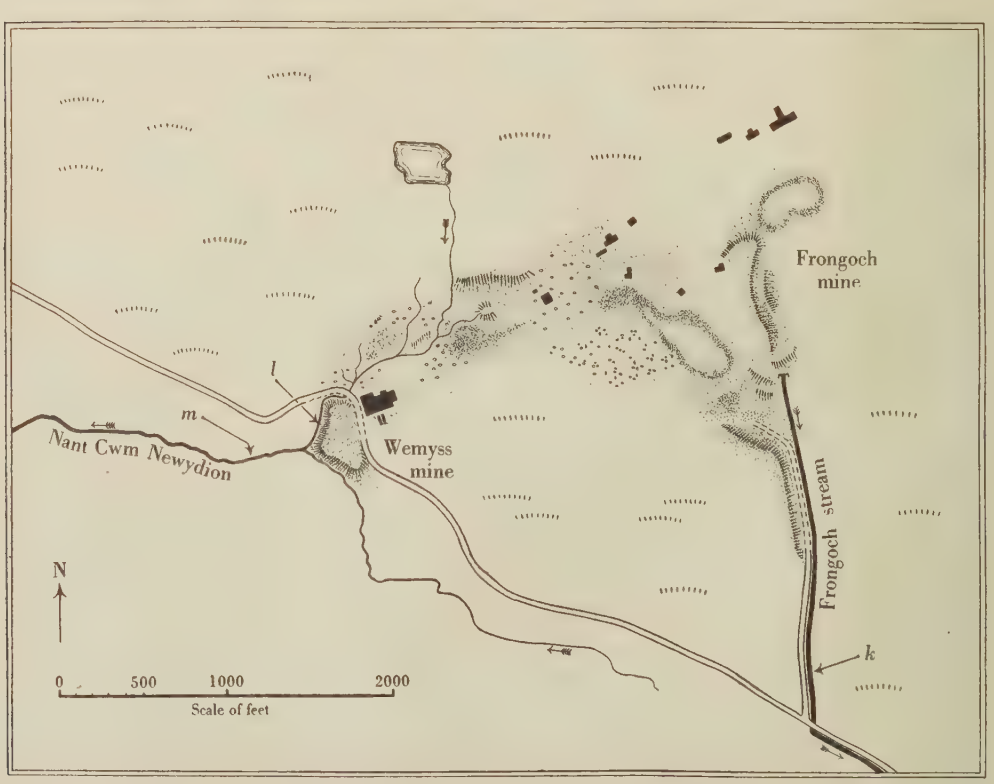

Fig. 4. The Frongoch and Wemyss mines showing the position of the Frongoch stream and the stream draining the surface of the Wemyss mine dump. Samples of water were taken at *k*, *l* and *m*. Key to symbols as Fig. 3.

TABLE 1. *Analytical data for the various sources of pollution of the river Ystwyth*

Concentrations are metal mg./l.

Source of pollution	*p*H	Zinc	Lead	Date
Cwm Ystwyth mine:				
Adit effluent (Fig. 3*a*)	6·4	38·0	8·0	11 Nov. 1939
Adit effluent (Fig. 3*b*)	5·6	32·0	1·5	,,
Stream draining dump (Fig. 3*c*)	5·6	0·8	0·35	,,
Adit effluent (Fig. 3*d*)	6·4	10·5	Trace	,,
Stream draining dump (Fig. 3*e*)	4·8	Trace	0·1	,,
Stream draining dump (Fig. 3*f*)	5·4	Trace	1·0	,,
Stream draining dump (Fig. 3*g*)	6·4	Trace	0·15	22 Nov. 1939
Stream draining dump (Fig. 3*h*)	6·2	Trace	Nil	,,
Frongoch mine:				
Frongoch stream (Fig. 4*k*)	5·8	57·0	3·0	16 Dec. 1939
Wemyss mine:				
Streamlet draining dump (Fig. 4*l*)	6·4	5·2	0·4	20 Dec. 1939
Nant Cwm Newydion (Fig. 4*m*)	6·4	3·2	0·2	12 Nov. 1939

(*c*) *The chemical condition of the mainstream*

A large number of determinations have been made of the zinc content and lead content of samples of the river water taken at various points and under various conditions. In Table 2 a representative series of figures is given for five samples of water from different points along the course of the mainstream. On this occasion the river was at normal level and the samples were all collected within a period of 2 hr. The figures show that pollution begins at the Cwm Ystwyth mine, below which the river is heavily polluted with zinc, and slightly polluted with lead. At Pontrhydygroes, above the Frongoch stream, the zinc content has been reduced somewhat, by the dilution effect of unpolluted tributaries; at station B the zinc content has been reinforced by the heavily polluted Frongoch stream, and has risen considerably, whereas the lead content has diminished to insignificant proportions. In the lower reaches of the river the unpolluted tributaries again exert a diluting action, and at station C the zinc content has fallen again, while lead is not present in measurable amount.

TABLE 2. *Representative analytical data for the mainstream of the river Ystwyth*

All samples taken 16 Dec. 1939. *p*H figures were determined colorimetrically with B.D.H. standard buffers. Concentrations are mg. metal/l. A blank space indicates no determination.

Station	*p*H	Zinc	Lead	Calcium
Above Cwm Ystwyth mine (Fig. 1F)	6·4	Nil	Nil	3·0
Below Cwm Ystwyth mine (A)	6·0	0·9	0·05	—
Above Frongoch stream at Pontrhydygroes (D)	6·2	0·7	—	3·7
Below Frongoch stream (B)	6·2	1·2	0·02	4·2
Llanfarian (C)	6·4	0·8	Trace	4·4

After heavy rain the volume of the river increases very considerably, while the major sources of pollution, being subterranean in origin, remain at much the same level. Consequently the zinc and lead content of the mainstream falls at flood-time, and analyses of filtered water samples taken at station C have shown that a normal flood is sufficient to reduce the zinc content, at this point, to 0·2–0·3 mg./l., and render lead indetectable. Conversely, when the level of the mainstream sinks below normal as a result of long spells of dry weather, the polluting effluents maintain their usual flow and the degree of pollution of the river increases. At such times the zinc content at station C may rise to 1·2–1·4 mg./l. These estimations refer to the amount of the metals carried in solution as salts: no estimations have been made of the amount of solid lead and zinc ores carried by the waters at flood time. Carpenter's experiments (1925) have shown that lead sulphide is not lethal to fish provided that it remains in suspension and does not pass into solution by being oxidized to lead sulphate. The solubility of lead sulphide is extremely low and that of zinc blende is only 0·4 mg. of zinc sulphide/l.

The river is still considered a source of danger to domestic animals and at the farms in its valley precautions are taken to prevent the animals visiting the river.

(*d*) *The flora*

The polluted mainstream, from the Cwm Ystwyth mine to Aberystwyth, is extraordinarily devoid of vegetation. There is a complete absence of aquatic phanerogams and the bryophytic vegetation is extremely scanty, being limited to small and occasional tufts of *Scapania undulata* growing at points where the river bed is rocky and shaded by trees. When the river is at normal or low level its margins are quite devoid of the submerged grasses usually found growing at the margins of rivers; the pasture grasses on its banks appear to survive temporary immersion at flood time (when the river is less heavily polluted), but permanent immersion, as may be occasioned by slight changes in the river's course, appears to cause them to wilt and rot rapidly. The microflora also appears to be very scanty and there is a conspicuous absence of the algal vegetation which is abundant in the unpolluted upper reaches of the mainstream and its unpolluted tributaries. In "cut-offs" only visited by the river at flood-time there is a sparse growth of *Callitriche aquatica* and *Glyceria fluitans*, and *Glyceria* is found growing at the mouths of certain tributaries, where the mainstream is diluted with unpolluted water. These localities, being unrepresentative, were avoided when taking fauna samples.

(*e*) *The fauna*

The fauna of the polluted reaches of the mainstream was studied by taking samples at three collecting stations, A, B and C, whose topographical features have already been described. The only collecting apparatus found necessary was a cheesecloth water-net about 18 in. deep with a semi-circular opening 12 in. in diameter. Large numbers of the submerged stones were overturned in the water and gently brushed with the hand so that the animals clinging upon and living beneath them were swept by the current into the net which was held immediately down-stream. This method proved very satisfactory, particularly for the capture of animals whose hold upon the stones is somewhat precarious and which are usually swept away and lost if the stones are lifted out of the water for examination, and, owing to the predominantly stony nature of the river bed and the complete absence of submerged grass, was responsible for the capture of the great majority of the specimens. Numbers of the stones were also examined carefully for the capture of animals not dislodged by this means. At each collecting station, on each occasion, approximately 40 min. was devoted to these collecting methods, the net being periodically emptied into a large jar containing about 2 l. of water. The net was also used to capture free-swimming Coleoptera and Hemiptera and to obtain samples of the dead leaves, twigs and other debris from the bottoms of pools; such material was placed in a separate collecting jar. The use of sieves was found unnecessary as all the pools in the polluted reaches of the river are carpeted with gravelly mine-dump refuse, which harbours no animal life, and there are no deposits of fine mud. A small bolting cloth net was used in the pools at stations B and C but this resulted only in the capture of very small and young specimens of species captured by other methods.

All the species recorded are listed in Table 3 which also indicates their relative abundance. A collection of aquatic Coleoptera identified by Prof. F. Balfour-Browne has been of great assistance in the identification of the beetles captured in the survey and in the identification of the Trichopteran larvae the work of Mr H. Evans of the Department of Zoology at Aberystwyth has been most valuable.

Table 3. *The fauna of the mainstream of the river Ystwyth*

The figures give the number of specimens captured at the collecting station, i.e. each is a sum-total for five fauna samples. When the number of specimens captured was between 100 and 500 the symbol *N* (numerous) is inserted. When the number exceeded 500 the symbol *VN* (very numerous) is used.

Collecting station ...	A	B	C
Collection dates ...	22 Oct. 1939	25 Oct. 1939	21 Oct. 1939
	22 Nov. 1939	21 Nov. 1939	14 Nov. 1939
	5 Dec. 1939	6 Dec. 1939	14 Dec. 1939
	13 Dec. 1939	11 Dec. 1939	20 Dec. 1939
	9 Jan. 1940	9 Jan. 1940	10 Jan. 1940
PLATYHELMIA			
Polycelis nigra Müll.	—	4	1
P. cornuta (Johnson)	—	—	4
Planaria alpina (Dana)	—	5	1
P. vitta Dugès	5	2	—
GORDIACEAE			
Gordius sp.	—	1	—
HYDRACARINA			
Hygrobates longipalpis (Herm.)	—	—	6
Diplodontus despiciens (Müll.)	—	1	—

TABLE 3 (*continued*)

Collecting station ...	A	B	C
Collection dates ...	22 Oct. 1939	25 Oct. 1939	21 Oct. 1939
	22 Nov. 1939	21 Nov. 1939	14 Nov. 1939
	5 Dec. 1939	6 Dec. 1939	14 Dec. 1939
	13 Dec. 1939	11 Dec. 1939	20 Dec. 1939
	9 Jan. 1940	9 Jan. 1940	10 Jan. 1940
PLEOCOPTERA			
Perla sp. *a*	4	—	—
Chloroperla sp. *a*	27	5	2
Perlodes sp. *a*	20	8	1
Leuctra sp. (or spp.?)	*N*	74	3
Isopteryx sp. *a*	28	14	43
Nemura sp. *a*	42	*N*	*N*
Nemura sp. *b*	*N*	6	1
Nemura sp. *c*	4	2	1
EPHEMEROPTERA			
Rithrogena sp.	*VN*	*VN*	*VN*
Ecdyonurus sp.	—	12	6
Chloëon sp.	—	*N*	*N*
Baëtis sp.	68	*N*	*N*
Leptophlebia sp.	12	—	8
HEMIPTERA			
Gerris lacustris L.	—	1	—
Velia currens Fab.	—	1	—
Notonecta glauca L.	—	9	10
Corixa geoffroyi Leach	—	—	1
C. striata Fieb.	—	1	1
C. sahlbergei Fieb.	—	1	—
Corixa sp. *a*	—	—	3
Corixa sp. *b*	—	2	8
TRICHOPTERA			
Rhyacophila dorsalis Curt. (bred out)	17	12	17
Polycentropus flavomaculatus Pict.	3	4	—
Plectrocnemia conspersa Curt.	—	32	—
Hydropsyche instabilis Curt. (bred out)	6	6	6
H. pellucidula Curt. (bred out)	15	3	2
Silo pallipes Fab. (bred out)	—	1	11
Philopotamus montanus Donov. (bred out)	2	—	—
Halesus radiatus Curt. (bred out)	—	6	14
Sericostoma personatum Spence (bred out)	2	7	6
Stenophylax stellatus Curt. (bred out)	1	6	1
Oxyethira sp. *a*	—	2	—
COLEOPTERA (imagines)			
Dytiscus marginalis L.	—	2	—
Agabus didymus Ol.	—	3	1
Platambus maculatus L.	—	2	—
Hydroporus palustris L.	—	—	1
H. lepidus Ol.	—	4	—
Deronectes elegans Pz.	—	—	1
Gyrinus natator L.	—	2	1
Helmis maugei Bed.	—	1	3
Latelmis volkmari Pz.	1	1	1
Riolus nitens Müll.	1	—	—
COLEOPTERA (larvae)			
Dytiscus semisulcatus Müll.	—	2	—
Illybius fuliginosus F.	—	—	6
Gyrinidae	1	4	3
Helodidae	1	1	3
Helmidae	1	1	—
Dryops sp.	1	1	5
DIPTERA			
Simulium sp. (or spp.?)	*VN*	*VN*	*VN*
Tipula sp.	—	1	1
Tabanus sp.	—	—	1
Tanypus sp.	3	1	—
Chironomus sp.	—	—	2
Dicranota sp.	—	—	1
Dixa sp.	—	—	1
Corynoneura sp.	—	—	4

Mr Evans has identified a number of the larval Trichoptera of the Aberystwyth district by rearing them to the adult stage. Such species are designated as "bred out" in Table 3. Five fauna samples were taken at each station. The collecting dates are given in Table 3.

(*f*) *Discussion*

Table 3 shows that the fauna of the polluted mainstream is of a very limited nature, being restricted to insects, Platyhelmia and Hydracarina. Vertebrata, Mollusca, Crustacea, Oligochaeta and Hirudinea are all unrepresented. The absence of fishes can be attributed to the dissolved zinc content of the water. The writer (1938) has shown that solutions of zinc sulphate in soft water are fatal to the stickleback, *Gasterosteus aculeatus* L., when the zinc content exceeds 0·3 mg./l. The survival time of mature sticklebacks in a 1·0 mg. zinc solution is about 34 hr., that of small specimens about 48 hr. The presence of small quantities of lead in addition to the zinc has no appreciable effect and solutions of lead salts alone, containing less than 0·1 mg. lead/l., do not appear to be fatal.

The rocks of the district are mainly Silurian grits and shales, are lacking in lime, and accordingly the water of the river has a low calcium content (see Table 2). The writer (1938) has shown that the toxicity to fishes of dilute solutions of lead salts and zinc salts is largely determined by the calcium content of the solution. Solutions of lead salts or zinc salts in soft water containing less than 5·0 mg./l. of calcium are considerably more toxic than solutions of equal lead or zinc concentration made up with hard water containing 40–50 mg./l. of calcium. *Gasterosteus* appears to survive indefinitely in a solution containing 2·0 mg./l. of zinc and 50 mg./l. of calcium (as nitrate, chloride or bicarbonate) but lives less than 24 hr. in a 2·0 mg. zinc solution in very soft water. Thus the river's low calcium content renders the metallic pollution more destructive to fishes.

Sewin occasionally ascend the river and a number have been caught by local anglers but the fish generally do not proceed beyond station C, where, it is reported, they are sometimes found dead or dying. Brown trout are plentiful in the mainstream above the Cwm Ystwyth mine and in many of the tributaries and are frequently carried into the mainstream at flood-time. They do not survive for long and are soon seen floating dead or helpless. The physiological nature of the toxic action of dissolved heavy metal salts on fishes has been discussed by Carpenter (1925, 1927), Ellis (1937) and Jones (1938, 1939).

The absence of Mollusca appears to be associated with the toxic nature of the river water. Davies (1938) has shown that a 1·0 mg./l. solution of zinc (as sulphate) is fatal to *Ancylastrum fluviatile* Müller in about 16 hr. and to *Limnaea pereger* Müller in 7 hr. There appears to be no experimental evidence available regarding the effects of zinc pollution on Entomostracan Crustacea, but Davies (1938) has shown that a 1·0 mg. zinc solution is fatal in about 57 hr. to *Gammarus pulex* (L.), a Malacostracan abundant in many of the unpolluted streams of the Aberystwyth district.

The insect fauna at all parts of the river is that of a swift stony stream and even in the lower reaches there is a singular dearth of species partial to still water. The vast majority of the river's inhabitants live upon or beneath the innumerable stones that form its bed and species generally found living on or in muddy bottom deposits and those that live among submerged vegetation are rare. Thus Plecoptera are numerous everywhere, and it is interesting to note that the stonefly *Nemura*, which is generally found living among water-weeds, has in the absence of submerged vegetation adapted itself quite successfully to living beneath stones.

In the Ephemeroptera there is a notable absence of mud-dwelling types like *Ephemera*, *Caenis* and *Ephemerella*, and the dominant mayfly is the flattened, stone-clinging *Rithrogena*. In the Trichoptera, larvae of the campodeiform type are the more common and there is a comparative scarcity of portable-case-carrying larvae. Finally the essentially lotic nature of the fauna is well illustrated by the *Diptera*; the stone-dwelling *Simulium* is the most abundant animal in the river; *Chironomus*, *Tanypus*, and other species partial to still water are rare.

There appears to be no evidence that the insect fauna is selectively limited by the zinc content of the water and its predominantly lotic nature is obviously associated with the singular physical nature of the stream; with its uniform swift flow, the stony nature of the river bed, and the lack of submerged vegetation. Even the heavily polluted Frongoch stream, containing nearly 60 mg./l. of zinc in solution, is not devoid of animal life. Half an hour's collecting at station E (Fig. 1) on 22 Dec. 1939 resulted in the following catch:

Nemura sp. *c*	3	*Halesus radiatus*	1
Leuctra sp.	37	*Plectrocnemia conspersa*	1
Rithrogena sp.	2	*Chironomus* sp.	1
Velia currens	2		

Welch (1935) emphasized that in running waters the grinding and scouring action of bottom materials set in motion by the current may cause much injury and mortality, the character and severity of this action varying with the force of the current and the nature of the bottom deposits. The Ystwyth is subject to frequent floods when its flow, even in the lower reaches, becomes violently swift and the stones on its bed are kept in continual motion at some points. After the flood has subsided it is noticeable that considerable changes have taken place in the distribution of the gravelly mine-dump material that litters the more placid regions of the stream-bed, and it is probable that at flood time the benthic fauna suffers severely from the grinding of the stones and the scouring action of the gritty deposits. It is possible that the rarity of planarians, and the absence of oligochaetes and leeches can be attributed, partly at least, to this factor; at least it seems reasonable to presume that these sluggish and soft-bodied animals are most subject to injury by molar action of the substratum.

The unpolluted upper reaches of the river are somewhat inacessible and no attempt was made to survey this part of the river in detail. The mainstream 1 mile above the Cwm Ystwyth mine was examined on 22 October and 5 December 1939 and brief investigation was sufficient to show that here it has every indication of being a healthy mountain stream. Trout are plentiful and the minnow (*Phoxinus phoxinus* L.) occurs in numbers. Small oligochaetes were found, two species of leech (*Herpobdella atomaria* Carena and *Haemopis sanguisuga* L.), the planarians (*Polycelis cornuta* and *Planaria alpina*) and the mollusc (*Ancylastrum fluviatile* Müller). There is an abundant algal and bryophyte flora and an insect fauna essentially similar to that occurring at station A, the most abundant species being stoneflies and campodeiform Trichopteran larvae.

The survey has shown that the drainage of water from open adits at disused zinc and lead mines may constitute a serious and very persistent source of pollution, sufficient to render a river barren of fish for very many years after the cessation of mining operations. The pollution caused by the drainage of surface water from exposed dumps of mine refuse is of a much less serious nature, is mainly effective at times of rain and flood, and may be so intermittent as to constitute little danger to fishes (Laurie & Jones, 1938). The destructive effect of lead

mining upon river fisheries has long been recognized; pollution by zinc mining has so far attracted little attention. This study may call attention to the fact that its effects may be equally serious.

Summary

1. The river Ystwyth, one of the main rivers of North Cardiganshire, has several disused metalliferous mine-workings in its valley, most of which have been idle for nearly 40 years. At certain of the disused mines, open adits continue to discharge water which is heavily polluted with zinc salts and to a less extent with lead salts. Pollution caused by drainage of surface water from exposed dumps of mine refuse also occurs but is comparatively unimportant. The mainstream of the river, when at normal level, carries 0·7–1·2 mg./l. of zinc in solution and a trace to 0·05 mg./l. of lead. The degree of pollution is temporarily diminished at flood time; increases when the level of the river falls below normal.

2. The river bed is silted up with vast quantities of broken rock, rubble and gravel carried down from the mine workings. The mainstream is completely devoid of phanerogamic vegetation and submerged grass and the bryophytic vegetation is extremely scanty.

3. The fauna of the mainstream is limited to Insecta, Platyhelmia and Hydracarina. The absence of fish is directly associated with the chemical condition of the water and the absence of Mollusca and Malacostracan Crustacea also appears to be due to the pollution. The nature of the insect fauna appears to be determined by the physical nature of the stream rather than by its chemical condition, the most abundant insects at all points being species characteristic of swift water and living upon or beneath stones. It is suggested that the rarity of planarians and the absence of oligochaetes and leeches may be due to the scouring action that the mine refuse and rubble on the river bed exerts upon sluggish soft-bodied animals.

4. Certain insect species appear to be very tolerant of zinc pollution and a number of species are found living in the most heavily polluted of the effluent streams.

5. The study calls attention to the fact that the destructive effect of zinc pollution upon river fisheries may be as serious as that caused by lead mining.

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THE MOISTURE BALANCE OF THE LIVING FLEECE IN RELATION TO BLOWFLY MYIASIS, WITH SOME REMARKS ON THE AUSTRALIAN AND BRITISH CONCEPTIONS OF SUSCEPTIBILITY

By JOHN MACLEOD

The Cooper Technical Bureau, Berkhamsted

It has long been appreciated, vaguely, that fleece moisture and sheep blowfly myiasis or strike are related, but until recently no attempt was made to define the precise mechanics of this relationship. Tillyard & Seddon (1933) reported that, for successful production of experimental strike, free water on the skin was essential, and suggested that the effect operated through a resultant increase of bacterial activity. Davies & Hobson (1935) showed that the fleece is normally an unsuitable environment for establishment of strike, on account of the unfavourably low air humidity at the base of the wool, and that only under abnormal conditions of high fleece humidity can eggs and young larvae survive and strike establish itself. Thus, for successful myiasis on healthy skin, the humidity must be over 90% for approximately 9 hr., to allow the eggs to hatch, and for a further period of at least 5 hr. of larval activity before a wound is produced capable of supplying the necessary moisture for larval survival. The humidity of the basal wool was found to be normally well below 70%, and the authors suggested that the fleece air humidity was, therefore, the limiting factor determining susceptibility to strike. Mackerras (1936) published a further summary of Australian investigations; he disagreed with the conclusion of the British workers, and stated that, under Australian conditions, actual free moisture on the skin and fleece, in addition to a high humidity, was necessary before strike could occur.

In an endeavour to explain the discrepancy between the Australian and British viewpoint, Lennox (1938*a*), in a study of the moisture affinities of the different constituents of the fleece, suggested that free moisture could occur in the fleece without the fibres being saturated, and that the apparently low fleece humidities recorded by Davies & Hobson were due to error introduced by this lag in equilibrium between free and absorbed moisture, since these workers had used the absorbed moisture content of the wool as an index of the microclimatic humidity.

Lennox's conclusions were:

(1) The content of absorbed moisture in the fleece depends on the relative humidity of the atmosphere, and not on its temperature.

(2) Free moisture may be present in a particular portion of fleece without the absorbed moisture content attaining to the equilibrium amount.

(3) After rain, dampness and sometimes water droplets occur irregularly in the body of the fleece.

(4) After rain, the basal wool, although generally drier than the distal, is wet to an extent dependent on the degree of wetting of the tip.

(5) Even after heavy rain, the moisture content of this basal wool is however less than that of wool in equilibrium with saturated air, i.e. the basal wool ordinarily does not have time

to come into equilibrium with saturated fleece air before rain ceases and the process of absorption is stopped by drying of the distal wool.

(6) The total moisture content of a fleece sample cannot therefore be used as a reliable index either of the relative humidity of the microclimate or of the suitability of the environment for blowfly maggot development.

Lennox's objection to the British theory seems to rest on the possibility that the fleece air may be saturated without the sampling method revealing this fact. It is difficult to conceive of free moisture remaining in the fleece without the microclimate becoming saturated, and indeed this saturation seems to be implicitly accepted in Lennox's argument. His findings do not, therefore, appear to invalidate the postulate that the fleece air must be over 90% saturated for strike to develop, but merely bring into question the conclusion that such a condition is as rare as the sampling results would indicate. Thus, the essential discrepancy between the British opinion that a saturated microclimate only is necessary for the development of strike, and the Australian contention that free water is necessary in addition to this saturated microclimate, remains unaffected by Lennox's contribution.

Two separate questions, therefore, arise for consideration. First, what is the actual humidity of a normal fleece climate, what is the moisture content of the fleece, and how are the two related? Does unequilibrated free water occur sufficiently often in the fleece in nature to justify a revision of the belief that the fleece is normally an unsuitable environment for establishment of strike? In other words, to what extent can Lennox's conclusions be confirmed? Secondly, what is the relative validity of the two divergent conceptions, the Australian and the British, of the role of moisture in the genesis of strike?

In the course of a more comprehensive study, which will be published later, of the general climate of the fleece in relation to season, an opportunity was presented of investigating the first question in some detail. The results and conclusions are presented below. It is with some misgivings that the writer has attempted, in the latter part of the paper, a critical review of the second question, in which it is suggested that the divergence of outlook is more apparent than real, and is due to a confusion of ideas; that, in fact, the evidence from both countries will bear a common interpretation.

The relative humidity of the basal fleece climate

Method. The relative humidity of the fleece air was determined by means of paper hygrometers. Calibration curves for 1, 2, and 4 hr. exposures at humidities of 10–90% were prepared; these were drawn as changes of log weight from that of 30 R.H. In this way the necessity for having the papers a standard weight was obviated. For all the experiments recorded below, a single batch of good quality white writing paper was used. The technique was as follows. Pieces of paper $2\frac{1}{2} \times 1\frac{1}{2}$ in., weighing approximately 400 mg. at 30 R.H., were stored at 30° C., 30 R.H., and always brought to the unknown humidity from this fixed starting point, because of the hysteresis effect in equilibrium from different humidity levels. Cylinders of brass gauze, $4 \times \frac{1}{2}$ in., closed at one end and with a gauze cap on the other were used for keeping the papers in the fleece. The papers were transferred from the standard 30° C., 30 R.H., system to tightly corked test tubes, in scrolls consisting of about $1\frac{1}{2}$ turns. In the field they were quickly transferred to the gauze cylinders, which were placed against the skin, in a natural "shed" of the wool. Two or three stitches of thread held the adjacent wool in place around them. After a fixed period of not less than 1 hr., and usually 2 hr., the end of each gauze cylinder was exposed, the cap removed and the scroll transferred by forceps very quickly into a test tube, which was tightly corked and brought to the laboratory. Weighings were made on a torsion balance, with a maximum load of 500 mg. and capable of being read to 0·5 mg. Tests showed that the short exposures, at transference from the fleece to the tube, and at weighing, did not appreciably alter the weight.

After weighing each paper was exposed for 24 hr. at 30 R.H. and again weighed, as a check for alteration of weight by soiling or other cause.

Experimental results. Table 1 gives the readings in the basal half inch of fleece for two crossbred sheep in April, under moderately warm conditions, with strong sunshine, and air humidities of 62 and 47% respectively. The observations, i.e. the intervals during which the hygrometers were left in the fleece, were $2\frac{1}{2}$ and $1\frac{1}{4}$ hr. in the early afternoon.

TABLE 1

Area	Sheep 1	Sheep 2	Area	Sheep 1	Sheep 2
Top of shoulder	37, 38	43, 43	Side of shoulder	38, 42	46
Mid-back	35, 40	—	Ribs	38, 39	41
Loins	41, 42	46	Side of thigh	39, 47	46
Tail head	45	37			

Table 2 gives comparative readings of three types of sheep: a half-bred, i.e. Cheviot-Leicester cross, with long open fleece, a Suffolk × half-bred cross, with moderately open fleece, and a Hampshire × half-bred cross, with relatively short wool. The first readings were made in early June, on a warm sunny afternoon, with an air humidity of 53–55% during the observation period; they were repeated on three similar sheep 18 days later, under almost identical weather conditions.

Numerous similar readings under summer conditions have confirmed the results given in Tables 1 and 2, namely that the air humidity in the basal fleece in summer is usually below 50%.

TABLE 2

	Half-bred		Suffolk cross		Hampshire cross	
Area	1st reading	2nd reading	1st reading	2nd reading	1st reading	2nd reading
Chest	50	36	42	41	41	55
Top of shoulders	45	33	39	39	40	33
Mid-back	39	—	41	—	37	—
Loins	38	28	35	37	37	35
Ribs	40	30	33	32	39	40
Side of thigh	—	40*	—	41	—	48

* Hygrometer partly exposed to outer air.

The theoretical humidity of the basal fleece. Under summer conditions, in the sun, the temperature of the skin may reach 40° C. In the body of the fleece there is usually a drop of greater or less extent depending on a variety of host and climatic factors, but on occasion the fleece temperature, through absorption of solar heat, may actually be higher than the skin temperature. Assuming an average temperature of 35° C. in the basal $\frac{1}{2}$ in. of fleece at 15 and 20° C., the limit of the air temperatures recorded in the above experiments, the vapour pressures in air 50% saturated are 6·4 and 8·8 mm. Hg. If there is no secretion of moisture in the fleece, so that the vapour pressure remains that of the outer air, the resulting humidities in the basal $\frac{1}{2}$ in., with a temperature of 35° C., would be 15 and 20%. The observed results were 35–50; i.e. it would appear that there is, in summer, a source of aqueous vapour in the fleece supplementary to the outer air. The nature of this source, and its possible identity with the "free water" of Lennox, were next investigated.

The water content of the fleece

The moisture content normally in or on the fleece components under dry summer conditions was estimated for different regions of the body of two crossbred sheep. The method used was to take samples of wool from areas approximately 2 in. square by cutting as closely as possible to the skin. Each sample was divided into a basal, mid and distal fraction, and these immediately weighed. The subsamples were then oven-dried to constant weight at a temperature of 90–100° C. Table 3 gives the water contents, expressed as percentages of the dried samples.

Table 3

	Sheep 1				Sheep 3			
Sampling region	Basal	Mid	Distal	Total staple	Basal	Mid	Distal	Total staple
Top of shoulders	9·4	6·1	5·02	7·05	12·46	8·91	7·76	9·8
Loins	9·75	4·6	4·44	6·24	9·24	4·34	5·74	6·43
Chest	13·15	13·4	10·6	12·6	18·58	18·95	15·25	17·9
Lower ribs	9·8	8·62	8·82	9·14	12·7	10	10·02	10·88
Side of thigh	14·3	10·45	11·25	11·82	9·27	14·27	14·3	12·56

Two points of interest arise from these results. In both sheep, the moisture content of the complete staple decreases in the following order: chest, thigh, ribs, top of shoulders, loins. Secondly, in the chest, thigh and rib samples there is slight or no decrease of water content from the base to the tip of the staple, but in the top of shoulder and loin samples a marked decrease. The water-soluble suint fraction, being the most hygroscopic, will at a given humidity above zero contain the largest relative amount of water. On the dorsal surface leaching by rain would be expected to keep the suint content of the distal third low. Little leaching will occur on the chest, lower ribs and side of thigh. The regional differences in relative moisture distribution along the staple can, therefore, be accounted for by leaching.

The fact that the basal third of the staple has the highest water content on the chest and the lowest on the dorsal surface would at first glance seem to be explicable on the same basis; it will be shown later, however, that rain does not ordinarily penetrate to the basal third, and it is possible that the actual secretion of suint is less along the back. This is supported by the finding that in winter, when activity of the skin glands would be expected to be low, there is a much less marked difference in the water content of the basal wool in different regions of the body.

In confirmation of the interpretation that the differential moisture content is related to the suint content, it is of interest that actual analysis of the suint distribution of Welsh sheep in summer (Hobson, 1936*a*) revealed the suint content to be relatively low on the back and high on the lower ribs and chest.

The relation between water content of the fleece and microclimatic humidity

Wool samples were taken from the backs of three crossbred sheep in May during a dry spell. They were weighed, then individually calibrated at known humidities, and the equilibrium humidities for their original weights obtained by interpolation. These were as follows:

Sheep ...	1	2	3
Basal third	62	68	66
Mid	59	62	58
Distal	74	78	71

The air temperature was 17·5° C., the air humidity 75 %. The humidity in the basal third of the fleece, if there were no secretion of moisture from the skin, and assuming the temperature of the basal wool to be 35° C., should, therefore, be 27 %. The absorbed moisture content of the basal wool is, therefore, in excess of that due to the humidity of the outer air, i.e. water is secreted from the skin. The question arises, therefore, is the microclimatic humidity in equilibrium with the water content of the fleece?

The readings obtained with paper hygrometers would suggest that this moisture was partly effective in raising the R.H. above that expected on the basis of no secretion. Unfortunately, no paper hygrometer readings were made in the case of the above sheep, so their actual fleece humidities are not known. It is highly probable, however, that these would not have been nearly so high as those calculated from the wool samples, since in no case in summer was a reading of over 50 % obtained on the back of a sheep in dry weather. It is almost certain, therefore, that the microclimatic humidity, although affected by the secreted moisture, is not in equilibrium with the water content of the basal wool.

Indirect evidence in favour of the above conclusion can be obtained as follows: If we assume a gradient of decreasing humidity with rising temperature from the tip to the base of the staple, the equilibrium R.H. found for the wool samples should decrease in a uniform fashion, since the outer air R.H. was the same for the three sheep, and their skin temperatures are approximately constant. The distal and mid-thirds show this uniform relationship, each sheep decreasing by 13–16° humidity. Were this gradient a straight line, the calculated R.H. for the basal thirds would thus be:

	Outer	Mid	Calculated inner	Actual inner
Sheep 1	74	59	44	62
,, 2	78	62	46	68
,, 3	71	58	45	66

The calculated values agree with the readings obtained by paper hygrometers in similar sheep (Tables 1 and 2). It would appear, therefore, that there occurs at the skin a secretion of moisture resulting in a lack of equilibrium between the basal fleece moisture and the microclimatic humidity. That is, there is a lag in equilibration which apparently disappears by the mid-third of the fleece. It is a justifiable assumption that this moisture is contained in the glandular secretions. If this theory be correct, winter conditions, which include a reduced secretion of yolk, might be expected to result in a marked reduction of this "lag". Actual test has shown that this is so. Thus in November, paper hygrometers were put in the fleece of a Suffolk cross ewe; after 1½ hr. they were removed, and simultaneously samples of the basal half to 1 in. of wool were taken from the same areas; these were weighed, and individually calibrated. The humidity, calculated from the hygrometers and from the wool samples, was as follows:

Top of shoulders:	R.H. reading of	4 paper hygrometers	30–35
	,,	4 wool samples	42–46
Loins:	,,	4 paper hygrometers	25–40
	,,	4 wool samples	34–44

There is here a fairly close agreement between the actual air humidity at the base of the wool and the theoretical humidity which would result in equilibrated wool having the water content possessed by the wool in question. That is, there has been a virtual disappearance of the "lag" found under summer conditions of skin-gland activity.

It would appear, therefore, that, under summer conditions, there is a higher water content of the basal fleece than would be indicated by the air humidity among that fleece; that this water, which occurs in the yolk as absorbed or bound water, causes a lack of equilibrium between fleece moisture and air moisture, and that equilibrium is restored in the middle of the fleece. These results suggest that, instead of there being a negative error, due to unequilibrated free water, as suggested by Lennox in the method of assessing humidity used by Davies & Hobson, there is, in fact, a positive error, due to unequilibrated absorbed water. That is, the actual humidities should be, if anything, somewhat less than those calculated by the above workers, and not higher values, up to saturation.

The effect of rain on the moisture balance of the fleece

Effect on water content. The penetration of rain into the fleece was studied in winter. It is to be regretted that this aspect of the work was not considered during the summer investigations, but, in view of the nature of the results, there does not appear to the writer to be any reasonable grounds for rejecting the applicability to summer conditions of the conclusions derived from the winter work.

A preliminary test of the normal moisture content of dorsal wool after several days of dry weather in winter gave the following result (Table 4) (water content expressed as percentages of the dried samples).

Table 4

	Sheep 1				Sheep 2			
	Basal	Mid	Distal	Total staple	Basal	Mid	Distal	Total staple
Top of shoulder	12·25	13·08	14·45	13·2	11·6	11·3	14·46	12·25
Loins	10·5	11·5	15·45	12·0	11·0	13·2	14·9	12·8

In November, after a day of drizzling showers, samples of wool were taken from the mid-back and ribs of an exposed sheep. The rainfall in mm. for the preceding 4 days, and for that day, was:

Day ...	4	3	2	1	0
	5·9	1·0	10·5	3·6	3·75

The sheep was a Suffolk cross ewe, relatively open-fleeced, with wool about 2 in. long.

Table 5

	Percentage moisture in the fleece			
	Basal third	Mid	Distal third	Total staple
Mid-back sample	12·65	16·15	66·7	31·3
Rib sample	11·45	17·4	20·85	16·55

This result shows that even on the dorsal surface of sheep exposed to several days' rain, the basal portion of the fleece is only very slightly affected, while the basal fleece of the ribs is scarcely affected at all. The tips of the dorsal wool have a high proportion of moisture,

mostly free water, apparent to visual and tactile appreciation, but the side wool is only slightly higher in water content than usual.

On the following day, rain again fell until 11.30 a.m., after which it remained dry, with a slight breeze. At 3 p.m. wool samples were taken from the mid-back of two sheep, a close wool Hampshire, with staple about 1 in. long, and a Suffolk cross, with open long wool fleece, about 2–2½ in. long. They were divided into two halves, an inner and outer.

TABLE 6

	% moisture		
	Inner half	Outer half	Total staple
Close-woolled sheep	18·45	68	43·3
Open-woolled sheep	12·36	36·4	23·9

The result of analysis of the open-woolled sheep conforms to that already obtained, with the interesting exception that the distal portion of the back wool, under the influence of about 3 hr. drying, has dropped markedly in its water content. With the close-woolled sheep, however, the position is markedly different. The outer half of the back wool is still very wet, and the basal wool moisture has increased perceptibly.* The high water content of the distal half may be due to there being an exceptionally high amount when the rain ceased, or, alternatively, to a reduced rate of drying in close wool.

It would thus appear that, even under conditions of heavy rain, the basal wool along the sheep's back does not become saturated. This is in agreement with the findings reported by Lennox, for dorsal wool of sheep after two days' rain.

Effect of rain on microclimatic humidity. It might be considered that, although the basal fleece does not absorb moisture to capacity during heavy rain, free water, present as a surface film on the staple or as droplets, might result in a saturated microclimate. It has been possible to show, however, that this saturation does not occur. Thus, in the case of the last experiment described, two paper hygrometer readings, taken simultaneously with removal of the wool samples (after 2 hr. exposure in the fleece), gave the following results for the basal loin wool:

Close-woolled sheep ... 62, 69.
Open-woolled sheep ... 39, 45.

In the case of the open-woolled sheep, where the water content of the basal wool was unaffected, the R.H. remained normal. An appreciable rise in R.H., but by no means to saturation point, in the fleece of the close-woolled sheep, is related, either as cause or effect, to the increased content of absorbed moisture in the fleece.

During May, in the afternoon of a day of drizzling rain, papers were put in the fleece of an exposed Suffolk cross sheep, and left for 3 hr. At the time of removal of the papers, the fleece tip felt wet on handling. The humidity readings were as follows:

TABLE 7

	Dorsal surface		Side
Top of shoulders	48, 52	Side of shoulder	52, 53
Mid-back	47, 73	Ribs	58, 69
Loins	55, 57	Side of thigh	41, 49
Tail head	43		

* Further work, to be published later, has confirmed that rain penetrates into close-woolled fleeces to a greater extent than into open-woolled fleeces.

The basal fleece did not approach saturation, although the outer surface was wet, and the outer air saturated.

The relationship between microclimatic humidity, fleece moisture, and outer air humidity

It has been shown that the absorbed moisture content of the fleece may be affected by other factors than the R.H. of the outer air, and, therefore, may vary independently of macroclimatic humidity. Variations in both factors, the fleece moisture and the macroclimatic humidity, are reflected in the humidity of the fleece air. What is the relationship between the three? Evidence has been obtained on this point (Table 8).

Table 8

		Fleece moisture % (loins)		
Sheep	Air humidity	Total staple	Inner half	Fleece humidity
A	70–80	43·4	18·45	60–70
B	70–80	23·9	12·36	40–45
C	100	16·55	14·45	70

Sheep A and B represent the relationship of microclimatic humidity and fleece moisture content, the outer air R.H. being constant, while sheep B and C represent the relationship of macroclimatic and microclimatic R.H., the fleece moisture content being approximately constant. It is clear that a causal relationship exists between the variables of each pair. It can be shown, however, that the fleece humidity is not proportional to either factor (Table 9).

Table 9. *Sheep D. Air humidity* 100

	Loins		Ribs	
	Water content	R.H.	Water content	R.H.
Basal third	12·65	70	11·45	50–60
Mid	16·15		17·4	
Distal	66·7		20·85	

Here, the fleece humidity is different for the back and ribs, although the macroclimatic humidity is the same, and although the moisture content of the basal and mid-fleece sections of the two regions is more or less equal. The two regions differ in the water content of the outer part of the fleece. It would thus appear that, in addition to air humidity, microclimatic humidity and water content of the adjacent wool, a fourth factor—the water content of the distal wool—enters into the complex of variables affecting the moisture balance at the skin. In the case above, the high water content of the distal wool was due to palpable free moisture in the wool tips, i.e. where free moisture is present in the fleece tips, the basal fleece humidity is increased beyond the point to which it is raised by saturated outer air. This may be due to the penetration of actual droplets of water to the inner portions of the fleece, once the distal fleece is loaded beyond saturation point; such droplets, reaching a microclimate with high temperature and relatively low aqueous tension, would rapidly evaporate, before they could be absorbed by the constituents of the inner fleece. This affords the only instance in this investigation of the possible role of free water in the basal fleece region. Such a result must be ephemeral, and can scarcely outlast the duration of the rain, for it will be quickly corrected by the equilibrium forces of the environment.

Discussion

It has been shown that the moisture content of the basal portion of the living fleece, although affected by the outer air humidity, is not proportional to this variable only. Secretion of moisture into or along with one component of the fleece, the yolk, possibly the suint fraction, is suggested as an independent source of moisture, under summer conditions. This affects the microclimatic humidity.

It has been suggested by Davies & Hobson (1935) that the increased absolute vapour pressure found at the skin, over that of the outer air, was due to evaporation from the skin. This would presumably originate from the sweat glands. There appears to be some doubt, however, Lennox (1938*b*), whether the coil glands in the sheep's skin produce true watery sweat. The suint secreted by them combines with the sebaceous secretions to form the yolk constituent of the fleece. Further, it has been shown in the present paper that the increase of bound moisture in the basal fleece over the equilibrium amount for the macroclimatic vapour pressure is greater than the absolute increase of vapour pressure in the basal microclimate over that of the macroclimate. These considerations support the conclusion that the microclimatic increase is but an incomplete reflection of the increase of moisture bound, or absorbed, in the fleece constituents, and not an independent accession from the skin.

Outer air conditions of actual precipitation, or conditions simulating the effect of precipitation on the fleece, such as wetting of the fleece tips by moisture-laden vegetation, or by casual accident, result in an excessively high moisture content of the distal wool, and this appears to exercise an independent effect on the microclimatic R.H. at the base of the fleece. The inter-relationships of the four variables, macroclimatic and microclimatic humidity, moisture content of the basal and of the distal wool, would thus appear to be highly complex.

Fundamentally, macroclimate affects the basal fleece microclimate, which in turn affects the absorbed moisture content of the basal fleece; secreted moisture, raising the water content of the basal fleece, reverses the causal relationship, and exercises a partial control of the microclimatic R.H., raising it slightly above the level determined by macroclimate. Equilibrium is not, however, established until about the middle of the fleece, i.e. at the source of moisture, the skin, a distinct lag occurs. Saturation of the outer air acts principally by increasing the absorbed moisture content of the distal fleece. This increases, probably by diffusion of absorbed moisture, the water content of the basal fleece, and so indirectly affects the basal fleece humidity. Excessive free moisture in the distal fleece, as with precipitation, may directly affect the basal R.H., i.e. independently of the basal fleece moisture content.

Whether this interpretation of the complex be correct or not, it is clear from the results that the absorbed moisture of the basal fleece, except as an ephemeral result of moisture-laden fleece tips, is never less than the equilibrium amount for the humidity conditions in the basal fleece air. That is, the R.H. of the basal fleece air, under none of the above conditions, will remain for any length of time greater than would be indicated by gravimetric calibration of the living fleece.

With regard to actual droplets of free water on the surface of the fibres, since these cannot remain unevaporated except in saturated air, and since, even under conditions of heavy rain, saturation of the basal fleece air does not result, their occurrence, except as a very ephemeral effect of rain, can be ruled out of serious consideration. It follows that newly hatched larvae at the base of the fibres must be dependent for their moisture supply on the aqueous

vapour of their environment, and will continue to be susceptible to the environmental humidity until exudation of moisture from the affected skin commences. The contention of Davies & Hobson that the R.H. of the fleece determines its suitability for the establishment of strike by young larvae is therefore justified, and the method of assessing this humidity by estimation of the moisture content of the fleece is reliable at least to the extent that it affords an index of the upper limit of humidity possible under the given conditions.

An analysis of the Australian and British theories of the immediate causes of strike

The Australian theories. From an early stage in the Australian investigations it was apparent that bacterial activity was associated with strike. Johnston (1923) and Seddon (1931) believed that bacterial activity in the wool was a necessary antecedent, and this theory was in part accepted by Holdaway (1933). Bull (1931) suggested that the role of bacteria was indirect, the ammonia produced by their activity causing a dermatitis in moist skin folds, which resulted in strike occurring.

In the Report of the Joint Blowfly Committee (1933), the development of attractiveness in an area of susceptibility is held to be, broadly speaking, "the result of moisture and bacteria acting on the skin itself, or on the wool, suint, yolk, and exudate".

The primary responsibility of moisture was postulated by Mackerras (1936), who stated, with regard to Merino sheep in Australia, "there is abundant evidence that the fleece must be distinctly and palpably wet through to the skin, and the skin itself must be wet, before a strike will occur.... It is not only necessary that the area to be struck should be wet; it must remain wet for a considerable period...." He summarized the arguments for and against an essential relationship between bacterial activity and strike, and concluded: "These last observations show definitely that the bacteria cannot be considered as of the same fundamental importance as water in the genesis of strike. They form an accessory condition, and the general evidence at least strongly suggests that they are an important accessory condition."

He again brings forward Bull's suggestion that dermatitis of the skin, produced by ammonia or by the bacteria associated with fleece rot, may be causally related to strike.

This dermatitis theory is again suggested by Belschner (1937): "As a general rule, under field conditions, it is not the wetness alone which attracts the fly to oviposit, but also the exudate resulting from the inflammation which follows the continual wetting by urine or water of an area of skin such as a crutch wrinkle." Like earlier writers, he comments on the almost invariable association of body strikes with bacterial fleece rot.

The Australian theories of the essential cause of strike on suitable sheep may thus be classified as follows:

(*a*) Wetness.
(*b*) Wetness + bacterial activity.
(*c*) Wetness + dermatitis from any cause.
(*d*) Wetness + bacterial activity resulting in dermatitis.

The British theory. This was first enunciated by Davies & Hobson (1935), who pointed out that (*a*) bacterial action, by producing decomposition odours, was essential for attraction of flies to oviposit, (*b*) high humidity was necessary for development of eggs and larvae. Since bacteria were normally present in the wool, moisture would thus lead to bacterial decomposition in the fleece. They claimed that the humidity at the base of the wool, the critical factor for survival of eggs and larvae, was therefore *the factor* which determined susceptibility to strike.

Hobson (1935, 1936*b*) showed that in the field, oviposition resulted only when the experimental sheep were made attractive by a variety of decomposition products, and in the following year MacLeod (1937) showed that eggs or larvae placed on the skin would establish strike on any sheep, and at any season, provided suitable moisture conditions were provided. It was thus clearly established that strike in nature was a dual phenomenon, consisting of two independent processes, the governing conditions for which might not necessarily be in harmony. This was further confirmed by MacLeod (1938), who reported the failure to survive of over 50 % of observed ovipositions, i.e. the conditions were suitable for oviposition but not for development of myiasis.

In the same paper, the writer elaborated the distinction between "blow", the deposition of eggs, and "strike", the development of a myiasis lesion, and suggested that, to avoid confusion, the term *susceptibility* should be restricted to that condition which rendered sheep liable to be blown: "As already stated, the establishment of strike, as distinct from the occurrence of blow is dependent entirely on the moisture conditions in the fleece, and so not affected by the susceptibility of the sheep. In other words, in a group of untreated sheep, exposed to conditions ideal for fly activity, only those which are susceptible will be blown, but if eggs are placed on the same group of sheep, under conditions of fleece moisture ideal for egg survival, all of the sheep will develop strike." It should be understood that, in the original enunciation of their theory, Davies & Hobson used the term susceptibility to mean suitability for larval development.

The British theory may be summarized as follows: Strike consists of two independent processes, blow, due to the existence of "susceptibility", and the development of myiasis, to which process the term strike in the restricted sense is applied. Susceptibility to blow results from the development of certain attractive odours on the sheep, among which ammoniacal and other decomposition products of bacterial activity are prominent. Strike results from the presence in the basal fleece of a high humidity, which enables the deposited eggs to hatch and the young larvae to establish a lesion on the skin.

It has been further suggested (MacLeod, 1937) that the establishment of strike is facilitated when the high humidity is limited to a small focus in the fleece, e.g. such as might result from the presence of a piece of extraneous hygroscopic matter; the young larvae on reaching the skin are thus prevented from scattering, their action is concentrated on a limited area of skin, and a lesion results more readily and with more certainty. This suggestion has not been tested experimentally, but forms the principle underlying the highly successful method of producing strike with eggs and larvae.

The differentiation of blow and strike. In considering the British and Australian view-points it is necessary to bear in mind that whereas the British workers distinguish rigidly between blow and strike, the Australians use the term strike comprehensively for both processes. Mackerras (1936) gives four stages in the development of strike, of which the first is the stage of attraction and egg laying. Similarly, the term susceptibility is used (Tillyard & Seddon, 1933) for the conditions allowing blow to occur and myiasis to develop: "For a sheep to be actually susceptible it must (*a*) attract the flies, (*b*) stimulate them to lay eggs, (*c*) provide the maggots with sufficient food for development."

This treatment of strike in the broad sense as a single process presupposes unified controlling factors, and has tended to obscure the fact that it is actually an ecological complex of essentially independent and possibly disharmonious processes. Thus, in marshalling the arguments for and against the role of bacteria, Mackerras (1936) gives as negative evidence, (*a*) the argument that not all sheep showing even great bacterial activity become struck in the field, and (*b*) that strike can be produced by placing maggots on a cleansed area, i.e. where bacterial numbers have been reduced below normal. These two arguments are only valid if strike be regarded in the restricted British sense, for in both the role of bacteria in producing blow is ignored. Yet in the same article, the arguments adduced as positive evidence refer to blow and strike indiscriminately. Thus (1) flies lay their eggs, and maggots grow in situations where bacteria are numerous and active (blow + strike); (2) following wetting and before the sheep become struck, there is great increase in the bacterial population of the wetted areas,—and this increase appears invariably to precede natural strike (either blow or strike might be intended here); (3*a*) the products of bacterial decomposition attract flies to oviposit (blow); (3*b* and 3*c*) bacteria liquefy animal matter and produce accessory food factors necessary for larval growth (strike).

In refusing to accept, for Australian conditions, Davies & Hobson's postulate, he says (p. 21): "Recently Davies & Hobson (1935) have studied the relative humidity of the air at the base of the fleece in Welsh sheep, and go so far as to say that this is 'the factor which determines susceptibility'. In Merino sheep in Australia, high relative humidity in the fleece is not alone sufficient; there is abundant evidence that the fleece must be distinctly and palpably wet through to the skin, and the skin itself must be wet, before a strike will occur.... It is not only necessary that the area to be struck should be wet; it must remain wet for a considerable period,—from two to eight or more days in our observations. During this period changes occur in the fleece and skin; they make the area highly attractive to *Lucilia* and frequently also specially suitable for invasion by its maggots. Even after the flies have laid their eggs, the area must remain wet long enough for the eggs to hatch and the young maggots to invade the skin a further period of about 24 hr."

Clearly, only the last sentence is germane to the argument: i.e. the restriction by the British workers of the term *susceptibility*, at this time, to the conditions allowing larvae to survive, although made clear by the context, has been completely overlooked. The same misunderstanding is patent in the

opening paragraph of Lennox's paper (1938*b*), which may be abbreviated as follows: "The moisture content of the fleece has long been known to influence susceptibility to blowfly strike. The opinion... of all Australian investigators is that the fleece and skin must be definitely wet,... supported by field and experimental observations...." (Here he cites Mackerras' evidence discussed above, i.e. evidence relating to the full myiasis sequence—attraction, oviposition, and larval development.) "*On the other hand*, Davies and Hobson... considered that the relative humidity of the microclimate at the base of the fleece was the important factor." The italics are the writer's, to emphasize the misconception.

The evidence for the role of free water in development of strike. If the evidence in favour of the necessity for palpable free moisture, as distinct from a saturated fleece atmosphere, is examined in the light of the foregoing remarks, it will be seen that none is valid when applied to strike in the restricted British sense, and that in practically every case its significance can be referred to the oviposition process. Further, the fact that artificial strike has been produced on cleansed shorn areas (Mackerras, 1936, p. 24) by wetting, and protecting the wetted area by a cellophane-covered pad of cotton wool, indicates that a saturated atmosphere is sufficient, for under such conditions, i.e. in the absence of wool and at a temperature of 35–40° C., the moisture left by wetting of the skin would not remain as an actual film of free moisture, available to the larvae, for the period of hours necessary for strike to develop.

That free moisture on the skin is highly unlikely to be often present in nature has been shown in the first part of this paper; that it is not necessary in the case of British breeds of sheep has been repeatedly proved by the writer, who has produced strike on Down breeds, on Kent, Suffolk, Ryeland, Border Leicester, and Cheviot breeds, by placing eggs or larvae, the latter on pieces of gauze cloth, on the skin, without free moisture, and placing a damp plug of cotton wool of about 1 in. diameter over them to provide highly humid air. Excess moisture was squeezed out of the plugs, so that free moisture could not be transferred to the skin from this source.

The role of moisture, bacteria and dermatitis in the initiation of strike. With regard to the significance of moisture in the causation of blow, the Australian evidence obtained in small cubicles, where the flies were crowded, is suggestive, but the evidence in larger cubicles, and in the field, does not rule out, but even suggests, the possibility that the effect of moisture may be indirect by allowing the production of decomposition odours through bacterial activity. Thus, although wetness is claimed to be the direct cause of the initiation of strike (i.e. of blow), it is admitted that, to be effective, it must persist for several days.

The role of bacteria is accepted by the Australians as probably important in the natural genesis of strike, and analysis of their argument shows that the mechanism of the effect may frequently be the production of putrefactive odours attractive to the fly. It is not claimed by Hobson that bacterial decomposition is the only source of attractive odours; thus, in this respect the two conceptions do not disagree.

The significance of ammonia and dermatitis is also a point on which no fundamental difference need exist between the two conceptions. The Australians tend to the belief that the sequence is: bacterial activity → ammonia → dermatitis → blow + strike. The British suggestion would be:

Summarizing this review, it may be said that fundamentally the evidence of the Australian and British schools on the genesis of strike agrees. Both accept the probability that in nature bacterial activity frequently initiates the myiasis sequence. The Australians believe that moisture is necessary as an antecedent to, and may be sufficient in itself without, bacterial activity; the British workers have offered no postulates for the relationship of moisture and bacterial activity, beyond the remark by Davies & Hobson that "there can be little doubt the presence of moisture will lead to bacterial decomposition in the fleece".

Given that the myiasis sequence is initiated, i.e. when eggs are laid, there is no significant divergence of evidence; both schools have shown the high susceptibility of incipient strike to frustration through drying, and the main difference is one of opinion as to the degree of moisture necessary for survival. The British state that saturation of the early larval environ-

ment is essential; the Australians accept this by implication but further postulate free water on the skin. The actual evidence from both countries merely reveals the necessity for saturation or almost saturation of the larval environment.

It is suggested, from the above analysis, that the apparent discrepancy between Australian and British findings rests less on a factual basis than on divergent conceptions of the essential structure of the problem, the evidence from both countries being in fact amenable to a common interpretation.

In conclusion, it seems reasonable to suggest that, since treatment of the myiasis sequence as a single problem is liable to lead, and has led, to confusion of ideas and argument, and must at the best result in a very complicated conceptual structure, progress would be facilitated by adoption of the method, current with British workers, of fractionating the problem into its component parts. Were this done, the further advantages would accrue that a standard terminology would become possible, and ideas and evidence would interlock more readily, with mutual advantage to those concerned in the investigations, and expedition of progress to the common ultimate goal.

Summary

The relative humidity of the air at the base of the fleece of close-woolled and open-woolled sheep was estimated gravimetrically by means of pieces of paper; it was found that under dry summer conditions the R.H. rarely exceeded 50% saturation.

The basal fleece was found to contain in summer a higher proportion of moisture than would fleece equilibrated with the adjacent air humidity; this is believed to be due to rapid secretion of moisture-containing yolk. Equilibrium between fleece moisture content and adjacent air humidity is re-established in the middle of the fleece.

Even under heavy or continuous rain the basal fleece on the dorsal surface of sheep rarely becomes saturated. Penetration is greater in close-woolled than open-woolled sheep. The basal fleece air does not become saturated during rain, but its vapour pressure may increase to some extent above normal if the moisture content of the basal fleece increases by diffusion down the fibres. Where the distal wool contains palpable free moisture in excess of its maximum absorbable amount, the basal humidity may be affected independently of the basal fleece content of absorbed moisture; this result is probably due to evaporation of water droplets which have penetrated to the inner fleece, and must necessarily be ephemeral.

Under normal conditions the R.H. of the basal microclimate must, for the above reasons, exercise a continuous inhibitory effect on the survival of newly hatched larvae, or of eggs laid in the basal fleece. Only under abnormal conditions will this factor permit their development, i.e. it is the limiting factor to establishment of strike.

Except during rain the total moisture content of the basal fleece affords a reliable index of the maximum humidity possible under the given conditions, and can, therefore, be used to assess the suitability of the area for development of strike.

In the second part of the paper an attempt is made to resolve the divergent outlooks of the Australian and British schools with regard to the immediate causes of strike. It is suggested that the apparent discrepancy of findings is based on a confusion of ideas, and that the evidence of the two countries is fundamentally in agreement.

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APHIS MIGRATION. AN ANALYSIS OF THE RESULTS OF FIVE SEASONS' TRAPPING IN NORTH WALES

By I. THOMAS AND E. J. VEVAI

School of Agriculture, University College of North Wales, Bangor

(With 5 Text-figures)

PROBLEMS concerning fluctuations in insect numbers due to climate and biotic factors have recently received particular attention by economic entomologists, and have also attracted the attention of mathematicians, who have generally dealt with fluctuations due to biotic factors, often disregarding the possible effects of climate. The inaccuracy of some of the conclusions drawn, and the theories of interactions of populations which have been propounded have been fully discussed by Thompson (1939). The importance of climatic factors as they affect insect populations has been emphasized by the work of Uvarov (1931). Climate (temperature, humidity and precipitation, wind, light and atmospheric pressure) affects insects directly and indirectly. The indirect effect is often of great importance because this involves the effect of climate on insect food and on other animals, including competitors and parasites and predators.

Fluctuations in numbers are directly affected by the conditions determining the flight of insects. These conditions, as they affect the flight of aphides, have been studied in some detail by Davies (1935*a*, 1936), who also constructed a water power mechanical insect trap (1935*b*) in order to obtain data on the subject in the field. This trap is a modification of the Williams & Milne (1935) electrically driven trap, and has now been working in North Wales for five successive years. In each year it has been set going towards the end of May, and daily records have been taken until mid-August. The records for 1935 and 1936 were obtained by the late Dr Maldwyn Davies. The insects were collected in celluloid tubes, which were changed daily at 9 hr. G.M.T. Although all specimens were preserved in alcohol, or lactic acid and alcohol, some were badly damaged; in such cases it was not possible to go beyond a generic determination. The classification adopted is after Börner & Schilder (1932) and Ris Lambers (1933, 1934) and the opportunity has been taken to clarify the position in this country as regards the nomenclature of certain aphides of economic importance.

In analysing the data obtained, certain anomalies appeared, and it became evident that, not only should the factors governing flight be considered, but also the factors influencing the production of alatae. An attempt has therefore been made to deduce the meteorological factors affecting wing bud formation. The meteorological data appertaining to temperature, relative humidity, wind velocity, rainfall, hours of sunshine and weather codes, were taken from the records of the meteorological station at the College Farm, Aber, near Bangor. The station is situated in a grass field about 200 yd. from the farm buildings, and the trap is in the same field.

The flight of aphides as affected by various climatic factors

Figs. 1–5 show the daily total catches of aphides for the period 1 June–15 July, 1935–9. Very few aphides were trapped before the beginning of June or after July in any year. The years 1935, 1936 and 1939 are regarded as "good years" for aphis migration, and the years 1937 and 1938 as "poor years". The meteorological factors influencing flight which are also

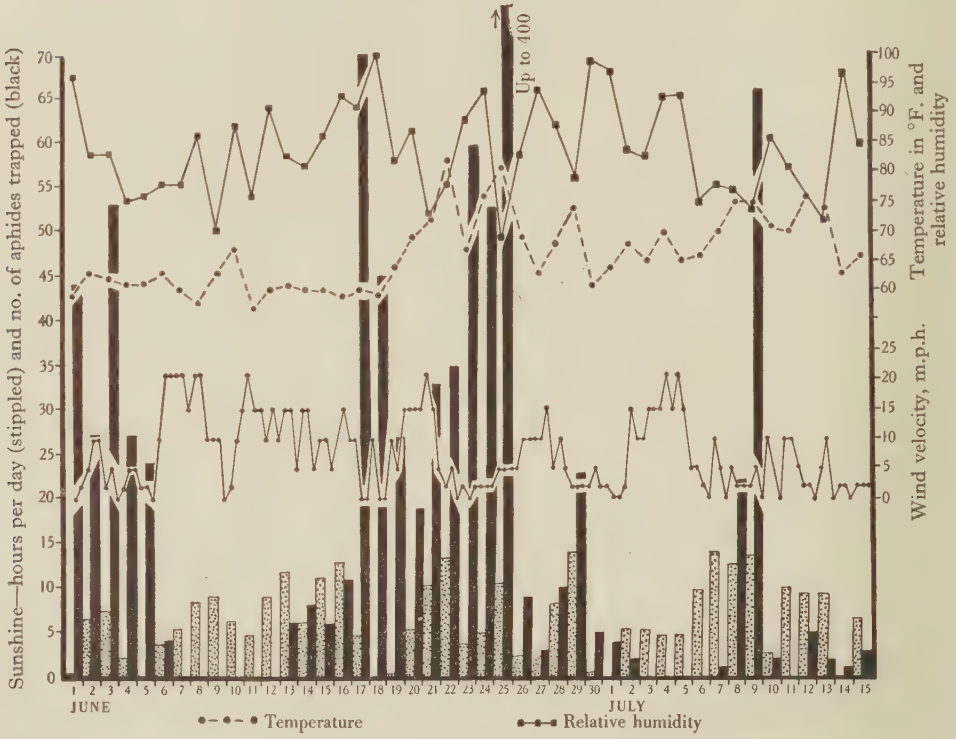

Fig 1. Meteorological conditions and aphides trapped during the migration period in 1935.

plotted are wind velocity, maximum temperatures, relative humidity and sunshine. The effects of each factor will be considered separately. Table 1 is a complete list of the aphides trapped in the five years, giving the full generic and specific names. The synonomy of some of the more important economic species is summarized in Table 2. Börner (1932) and Ris Lambers (1933, 1934) have adopted a different view from Theobald (1926) with regard to the synonomy of a few of the species listed and the name adopted by Theobald has, therefore, been put in a separate column.

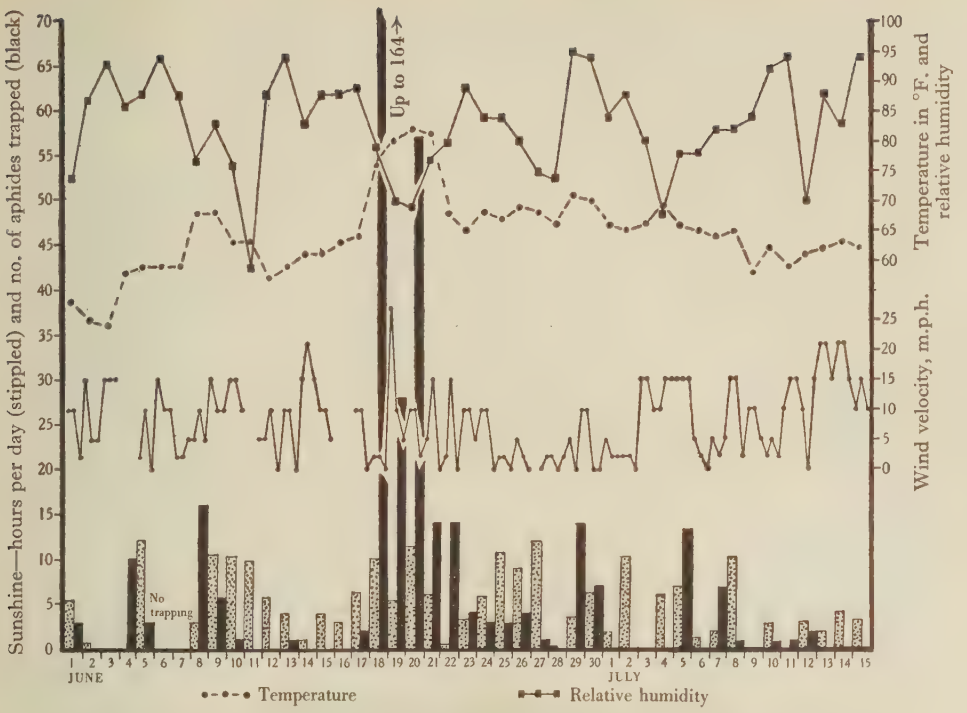

Fig. 2. Meteorological conditions and aphides trapped during the migration period in 1936.

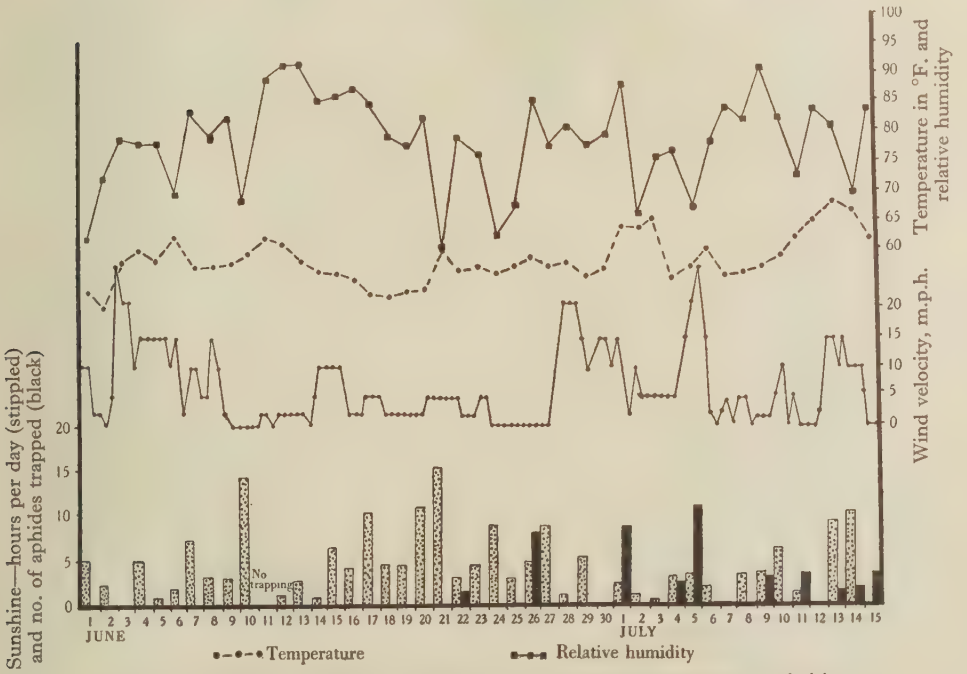

Fig. 3. Meteorological conditions and aphides trapped during the migration period in 1937.

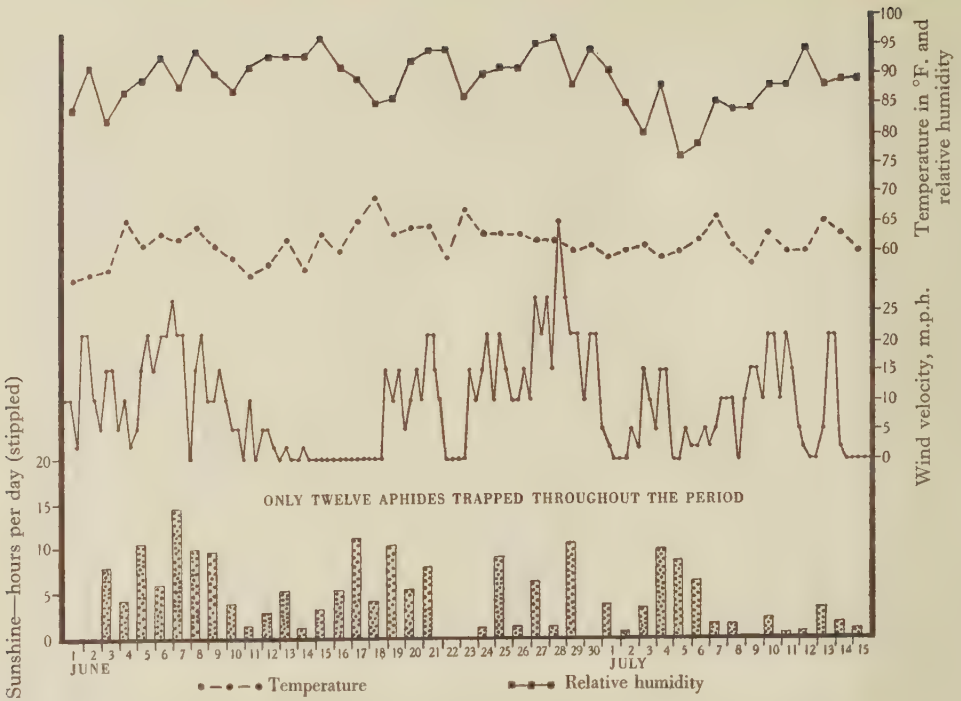

Fig. 4. Meteorological conditions and aphides trapped during the migration period in 1938.

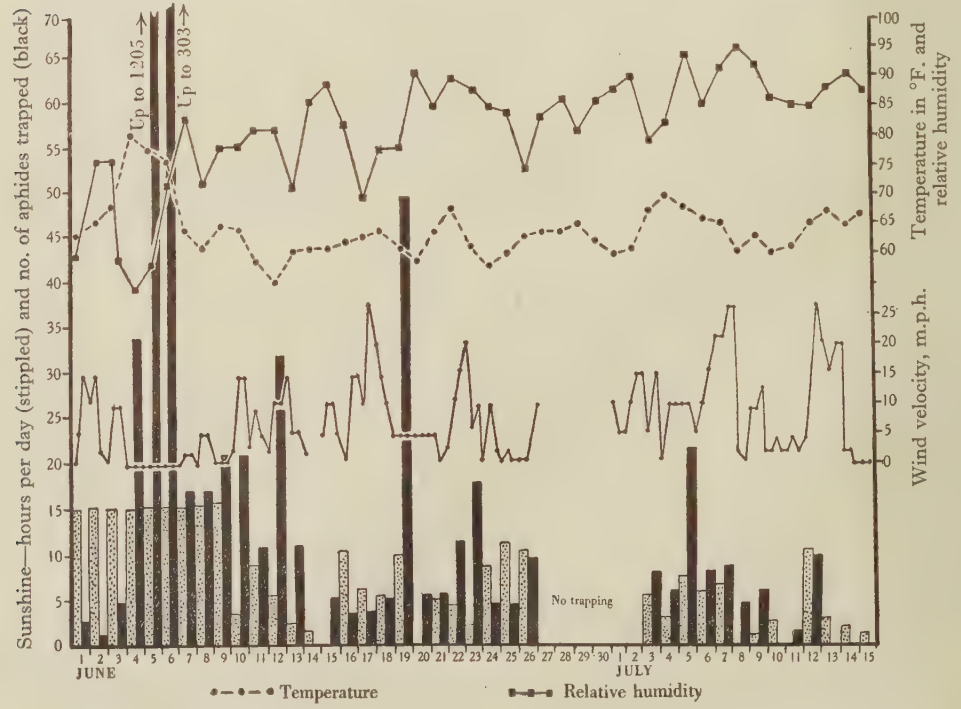

Fig. 5. Meteorological conditions and aphides trapped during the migration period in 1939.

TABLE 1. *The species and number of aphides trapped in the years 1935–9*

	Species	Number trapped 1935	1936	1937	1938	1939
1.	*Saltusaphis ornatus* Theob.	2	—	—	—	17
	Saltusaphis sp.	3	—	—	—	–
2.	*Euceraphis betulae* L.	1	—	1	—	—
3.	*Phyallaphis fagi* L.	1	–	–	—	—
4.	*Drepanosiphum platanoides* Schr.	11	13	—	1	2
5.	*Myzocallis castanicola* Baker	2	—	—	—	2
6.	*Myzocallis annulata* Hartig.	—	—	—	—	7
	Myzocallis sp.	—	—	—	—	1
7.	*Sipha glyceriae* Kalt.	—	2	—	—	–
	Sipha sp.	1	—	–	—	—
8.	*Periphyllus aceris* Koch	—	—	—	—	1
	Periphyllus sp.	—	—	—	—	1
9.	*Brachycaudus helichrysi* Kalt.	305	68	—	2	347
10.	*Brachycaudus cardui* L.	1	3	—	—	8
	Brachaudus sp.	—	2	—	—	2
11.	*Yezabura malifoliae* Fitch	4	—	—	—	–
12.	*Hyalopterus arundinis* Fab.	146	22	15	—	20
13.	*Hyalopterus atriplicis* L.	1	—	—	—	–
	Hyalopterus sp.	—	2	–	–	—
14.	*Rhopalosiphum padi* L.	30	11	—	4	90
15.	*Rhopalosiphum crataegellum* Theob.	16	8	—	—	21
16.	*Rhopalosiphum nymphaeae* L.	3	—	–	—	—
	Rhopalosiphum spp.	—	2		—	–
17.	*Aphis sambuci* L.	8	1	6	1	5
18.	*Aphis plantaginis* Schr.	1	—	—	—	1
19.	*Aphis triglochinis* Theob.	2	—	—	—	–
20.	*Aphis beccabungae* Koch	2	—	–	—	–
21.	*Aphis newtonii* Theob.	2	1	—	—	4
22.	*Doralis frangulae* Koch	3	1	1	—	—
23.	*Doralis laburnii* Kalt.	1	—	—	–	—
24.	*Doralis fabae* Scop.	4	1	6	1	24
25.	*Doralis symphyti* Schr.	5	1	—	–	—
26.	*Doralis urticaria* Kalt.	8	2	–	—	–
27.	*Doralis rumicis* L.	1	—	1	—	2
28.	*Doralis tormentillae* Pass.	—	—	1	—	—
	Doralis spp.	84	2	2	—	6
29.	*Hyadaphis sii* Koch	1	1	1	—	76
30.	*Myzaphis rosarum* Kalt.	1	—	—	—	9
31.	*Hyalopteroides dactylidis* Hay.	—	—	—	—	135
32.	*Cavariella aegopodii* Scop.	31	95	2	—	570
33.	*Cavariella* sp. (with sensoria on 4th segment of antenna)	1	43	—	—	12
34.	*Cavariella pastinaceae* L.	85	67	2	—	41
35.	*Cavariella umbellatarum* Koch	134	19	—	—	20
	Cavariella spp.	3	5	6	6	43
36.	*Neomyzaphis abietina* Walk.	—	—	—	—	3
37.	*Capitophorus elaegnii* Del Guercio	—	—	1	—	—
38.	*Capitophorus similis* V.d.G.	—	1	—	—	4
38*a*.	*Capitophorus hippophaes* Walk.	2	–	—	–	—
	Capitophorus spp.	2	2	1	—	7
39.	*Phorodon menthae* Buckt.	2	—	—	—	—
40.	*Phorodon humuli* Schr.	2	2	—	—	49
41.	*Phorodon crataegarium* Walk.	—	—	1	—	17
	Phorodon spp.	2	—	—	1	—
42.	*Cryptomyzus ribis* L.	2	—	—	—	3
43.	*Cryptomyzus galeopsidis* Kalt.	3	—	—	—	18
44.	*Pentatrichopus potentillae* Walk.	—	1	—	1	—
45.	*Trilobaphis caricis* Theob.	2	—	—	—	—
46.	*Myzus persicae* Sulz.	55	2	3	1	9
47.	*Myzus cerasi* Fab.	10	3	—	1	4

TABLE 1 (*continued*)

	Species	Number trapped 1935	1936	1937	1938	1939
48.	*Myzus ornatus* Laing	—	—	—	—	1
	Myzus spp.	2	—	—	—	—
49.	*Ovatus mespili* V.d.G.	4	5	2	—	1
	Ovatus spp.	1	—	1	—	1
50.	*Rhopalosiphoninus staphyleae* Koch	5	4	1	—	11
51.	*Rhopalosiphoninus calthae* Koch	—	—	—	—	1
52.	*Rhopalosiphoninus lactucae* L.	1	1	—	1	4
53.	*Rhopalosiphoninus rhinanthi* Sch.	8	4	1	—	4
54.	*Rhopalosiphoninus rubi* Kalt.	—	—	—	—	1
	Rhopalosiphoninus spp.	—	2	1	—	1
55.	*Acyrthosiphon onobrychidis* B.d.F.	5	—	2	1	6
56.	*Acyrthosiphon carnosum* Buckt.	43	—	—	—	10
57.	*Acyrthosiphon loti* Theob.	—	—	—	—	1
58.	*Aulacorthum solani* Kalt.	3	—	—	—	5
59.	*Aulacorthum pelargonii* Kalt.	1	3	—	—	2
60.	*Nasonovia ribicola* (*hieracii*) Kalt.	7	1	4	4	11
61.	*Metopolophium dirhodum* Walk.	7	4	1	6	35
62.	*Megoura viciae* Kalt.	—	—	3	—	—
63.	*Macrosiphum solanifolii* Ash.	6	—	—	1	—
64.	*Macrosiphum rosae* L.	3	3	2	1	3
65.*	*Macrosiphum rubiellum* Theob.	24	17	—	—	17
66.	*Macrosiphum* (*Sitobion*) *avenae* Fab.	15	—	—	—	—
67.	*Dactynotus* (*Uromelan*) *jaceae* L.	4	—	—	—	4
68.	*Macrosiphoniella sejuncta* Walk.	—	—	—	—	2
69.	*Vacuna dryophila* Schr.	—	1	—	—	283
70.	*Anoecia corni* Fab.	5	2	—	1	2
	Pemphigus spp.	2	—	—	—	—
71.	*Thecabius affinis* Kalt.	5	—	—	—	1
72.	*Eriosoma lanigerum* Haus.	9	—	—	—	—
73.	*Eriosoma lanuginosum* Hartig	2	—	—	—	—
74.	*Eriosoma ulmi* L.	4	—	—	—	2
	Eriosoma spp.	4	3	—	1	1
75.	*Tetraneura ulmifoliae* Baker	—	1	—	—	—

* Ris Lambers in his "Contributions to a Monograph of the Aphididae of Europe", *Teminckia*, 4, 113, considers this species to be a synonym of *Macrosiphum* (*Sitobion*) *fragariae* Walk.

(*a*) *Wind velocity*

The wind velocity was recorded at the station at 9, 15 and 21 hr. G.M.T., and was measured in terms of Beaufort's scale for wind force. There is a range of wind velocities for each Beaufort number above 1, and for the purpose of plotting the graphs the mean value in miles per hour has been adopted. Figs. 1–5 show that the great majority of aphides flew at wind velocities below 5 m.p.h.; this is in keeping with Davies' (1936) experiments in the laboratory. The aphides trapped at the various wind velocities for the years 1935, 1936 and 1939 are recorded in Table 3. There is a sudden fall in the number of aphides trapped when the wind velocity rises above 1 m.p.h. The peak migration days in the good years had a wind velocity which was invariably below 5 m.p.h. It must be realized that there might have been fairly long lull periods between the recorded velocities, and therefore Table 3 does not give a perfect correlation of wind velocities with numbers of aphides trapped. Such lull periods might even have occurred when aphides were trapped on days when all the recorded wind velocities were above 5 m.p.h. The velocity charts for every day were therefore examined, and it was found that there were only 11 days in the 5 years when the velocity never fell

TABLE 2. *The more important synonyms of some aphides recorded in Table 1*

Modern nomenclature (after Börner and Ris Lambers)	Main description of Theobald under the name	Important synonyms
Rhopalosiphum crataegellum Theob., oat apple aphis	*R. prunifoliae* Fitch	*crataegi* Buckt. non Kalt. *crataegella* Theob. *Fitchi* Börn. non Sanderson
Rhopalosiphum padi L. (Kalt. Koch), European grain aphis	*Aphis avenae* Fab.	*prunifoliae* Fitch partim ? *pseudo-avenae* Patch
Rhopalosiphum annuae Oest., American grain aphis	It is doubtful whether this species occurs in the British Isles	*prunifoliae* Fitch partim (Baker & Turner) *Fitchi* Sanderson *rufiabdominalis* Saski. *oryzae* Matsum.
Nasonovia ribicola Kalt.	*Myzus lactucae* Schrk.	*hieracii* Kalt. *lactucae* Schrk. (preoccupied by *R. lactucae* L.)
Macrosiphum (*Sitobion*) *avenae* Fab.	*M. granarium* Kirby	*granaria* Kirby *alii* Jackson *Macrosiphoniella triglochiniella* Theob. *Aphidiella secretocauda* Theob.
Brachycaudus helichrysi Kalt.	*Anuraphis helichrysi* Kalt. (Theobald considered *Aphis padi* L. to be a synonym, II, p. 403)	*sherardiae* Theob. *achilleae* F. (of Theob.) *crithmi* Buckt. *tanacetina* Walk. (of Theob.) *instabilis* Buckt. *verbenae* Macchiati (of Theob.) *bellis* Buckt. *Neoacaudus bipapillata* Theob.
Doralis symphyti Schr.	*Aphis rhamni* B.d.F.	*rhamni* B.d.F. *nasturtii* Kalt. *gossypii* Glover *solanina* Pass.
Aulacorthum solani Kalt.	*Myzus pseudosolani* Theob. (In III, p. 338, Theobald considers this to be a synonym of *solani* Kalt.)	*pseudosolani* Theob. *glauci* Theob. *mercurialis* Theob. *polyanthi* Theob. *aquilegiae* Theob. *neogei* Theob. *duffieldii* Theob. *polygoni* Buckt. *piceaellus* Theob. *convolvuli* Kalt. *hydrocotylei* Theob. *chelidonii* Kalt.
**Macrosiphum solanifolii* Ash.	*M. gei* Koch partim	*epilobiellum* Theob. *hellebori* Theob. & Walton *amygdaloides* Theob. *centranthi* Theob. partim *sileneum* Theob. *fragariae* Buckt. *euphorbiellum* Theob. *hibernaculorum* Theob.
Macrosiphum cholodkovskyi Mord.	*M. gei* Koch partim	*ulmariae* Theob. partim
Macrosiphum gei Koch	*M. gei* Koch partim	*corallinum* Theob.
Nasonovia ribicola Kalt.	*Myzus lactucae* Schr.	*lactucae* Schr. *hieracii* Kalt.
Acyrthosiphon onobrychidis B.d.F.	*Macrosiphum pisi* Kalt.	*pisi* Kalt. *theobaldi* Davis
Rhopalosiphoninus staphyleae Koch	*R. tulipaella* Theob. ? *R. latysiphon* Davis	*R. tulipaella* Theob. *R. lupulinus* Theob. *R. pseudorumicis* Theob. *R. waltoni* Theob. & Walton
Rhopalosiphoninus lactucae L.	*Amphorophora cosmopolitanus* Mason	*A. cosmopolitanus* Mason

* Ris Lambers in his "Contributions to a Monograph of the Aphididae of Europe", *Teminckia*, **4**, 84, considers this species to be a synonym of *M. euphorbiae* Thomas.

below 5 m.p.h. During these days a total of only 27 aphides were trapped, of which 16 were *Brachycaudus helichrysi*. The others included *Drepanosiphum platanoides* (2); *Macrosiphum* (*Sitobion*) *avenae* (2); *Macrosiphum rubiellum* (2); and single specimens of other species.

Davies (1936) pointed out that wind direction is also an important factor. Farmers often complain that greenflies and other insects come with an east or north-east wind. Under the conditions in Minnesota, Ainslie (1926), when referring to outbreaks of *Toxoptera graminum* Rond. states that the aphides rose into the air only when the wind was from the south.

TABLE 3. *The number of aphides trapped at various wind velocities during the seasons* 1935, 1936 *and* 1939. *The velocity quoted is the mean of the three recordings in any day*

Wind velocity in m.p.h.	No. of trapping days	Total aphides	Wind velocity in m.p.h.	No. of trapping days	Total aphides
0	3	1538	10	11	83
1	3	1330	11	2	32
2	11	305	12	7	71
3	8	141	13	2	2
4	3	46	14	2	61
5	6	478	15	4	49
6	3	20	17	1	4
7	10	137	18	1	10
8	7	91	25	1	8
9	2	8	29	1	4

An examination of the wind direction charts revealed:

(i) That the peak migrations of the *good years* fell exclusively on days when the wind was from the north-east.

(ii) That in the *good years* in the majority of cases the wind was from the south-west (41·7% in 1935, 32·4% in 1936, 21·7% in 1939). There were comparatively few cases when the wind was from the west (3·8% in 1935, 4·1% in 1936, 20·2% in 1939), and there were a considerable number of cases when the wind was from the north-east (16·7% in 1935, 10·6% in 1936 and 11·2% in 1939).

(iii) In the *poor years* in the majority of cases the wind was from the west (28·2% in 1937, 46·2% in 1938). There were comparatively few cases of wind from the south-west (7·4% in 1937 and 6·1% in 1938), and very few cases of wind from the north-east (4·5% in 1937 and 2·2% in 1938).

(iv) In *all years* south-west winds were strong with velocities generally above 5 m.p.h., whereas the velocities of north-east winds were usually below 5 m.p.h.

(*b*) *Temperature*

In laboratory experiments, Davies (1935*a*) showed that at temperatures below 55° F. *Myzus persicae* Sulz. is reluctant to spread out its wings; at 70° F. it flies well, and better still at temperatures up to 90° F. Table 4 shows that a number of aphides were trapped at temperatures below 75° F., but by far the greatest number flew at temperatures above this level. The peak migration days all had maximum temperatures above 75° F. From the temperature charts, it also appeared that the greater the difference between the daily maximum and minimum temperatures, the greater was the number of aphides flying. Out of the 3355 aphides trapped in 1935, 1936 and 1939, 2389 were trapped on only 13 days with temperatures above 75° F. Of the aphides trapped at temperatures below 60° F., 95 were

Brachycaudus helichrysi; other prominent species were *Myzus persicae* (25); *Rhopalosiphum padi* (20); *Hyalopteroides dactylidis* (18); *Cavariella pastinaceae* (16); *Acyrthosiphon carnosum* (16); *Macrosiphum rubiellum* (9) and *Rhopalosiphum crataegellum* (17).

TABLE 4. *The number of aphides trapped at daily maximum temperatures in the seasons* 1935, 1936 *and* 1939

Max. temp. in ° F.	No. of trapping days	Total aphides trapped	Max. temp. in ° F.	No. of trapping days	Total aphides trapped
82	2	101	66	3	22
81	2	412	65	5	44
80	2	62	64	7	72
78	1	1203	63	8	56
76	4	523	62	5	92
75	2	88	61	8	89
74	3	26	60	7	139
73	1	1	59	7	121
72	1	33	58	2	18
71	2	16	57	1	1
70	3	10	55	1	32
69	3	32	53	1	3
68	10	88	50	1	0
67	3	71	49	1	0

(*c*) *Relative humidity*

Davies (1935*a*) showed that the flight of *Myzus persicae* was affected by changes in the relative humidity only when the temperature rose above 70° F. Figs. 1–5 show that on all the peak days for aphis catches the relative humidity was low, but aphides were trapped in considerable numbers even at relative humidities above 90. There were only 2 days with very low relative humidities of 57–58, but on these two days 1237 aphides were trapped (Table 5).

TABLE 5. *The number of aphides trapped at various ranges of relative humidities in the seasons* 1935, 1936 *and* 1939

Relative humidity	No. of trapping days	Total aphides trapped
57– 58	2	1237
69– 70	5	498
71– 80	31	937
81– 90	37	394
91–100	17	291

(*d*) *Sunshine and precipitation*

Both these factors affect flight indirectly because of their effect on temperature and humidity. A number of aphides were taken on days when the total rainfall was above $\frac{1}{2}$ in., but it is possible that there were dry periods even with such a high rainfall. Aphides prominent in the catches on such days were *Brachycaudus helichrysi*, *Cavariella pastinaceae*, *C. umbellatarum* and *C. aegopodii*. All the peak catches occurred on days of over 10 hr. sunshine, but a total of 100 aphides were caught on 9 days when no sun was recorded. 534 aphides were trapped on 38 days with 0–5 hr. sun, 507 were trapped on 31 days with 5·1–10 hr. sun, and 2233 were trapped on 30 days with 10·1–15·5 hr. sun.

The flight of aphides in different years

Davies (1935*a*, 1936) found that the optimum conditions for flight of *Myzus persicae* were a temperature above 70° F., a relative humidity below 70, and a wind velocity below 4 m.p.h. Taking into consideration the data presented above, and in particular the conditions obtaining during the days of peak migrations, the following arbitrary standards are suggested for the flight of aphides in general:

(*a*) A temperature above 70° F.

(*b*) A relative humidity below 80.

(*c*) A wind velocity below 5 m.p.h.

(*d*) A big difference in maximum and minimum temperatures (more than 12° F.).

The meteorological data for the month of June in each year have been analysed, bearing in mind these arbitrary standards.

(*a*) *June* 1935 (Fig. 1). In June 1935 there were 10 days with a temperature above 70° F., and a relative humidity below 80; there was a very wide fluctuation in daily temperatures: the sunshine averaged 6·64 hr. per day, and there were long periods when the wind velocity was below 5 m.p.h. A considerable number of aphides were trapped in this year.

(*b*) *June* 1936 (Fig. 2). This June was the coolest of the "good" years; there were 5 days with a temperature above 70° F., and the average daily fluctuation in temperature was 13·3° F. There were only 3 days when the relative humidity fell below 80, but the wind velocity was below 5 m.p.h. for long periods. The sunshine averaged 5·4 hr. per day. On the whole, one would not have expected this month to have been a good one for the flight of aphides, and in fact catches were confined to a few days.

(*c*) *June* 1937 (Fig. 3). On the average, this June was warmer than in 1936, but, on the other hand, the temperature did not rise above 70° F. on any day, and there were only 8 days with a relative humidity below 80. Fig. 3 shows that 1937 was a "poor" year for aphis migration, but there were occasional days when large numbers were expected in the trap. The presence of so few aphides may have been due in part to the scarcity of alatae.

(*d*) *June* 1938 (Fig. 4). This was the poorest of all the years, only twelve aphides being trapped even up to 15 July. The temperature did not once rise above 70° F. The relative humidity was abnormally high; it never fell below 80 and was frequently over 90. The total rainfall was 4·87 in.

(*e*) *June* 1939 (Fig. 5). 81 % of the total aphides trapped in 1939 were taken on the only 3 days in June which had a temperature of over 70° F. This month was outstanding for being very dry; there were 14 days with a relative humidity below 80, and the sunshine averaged 8·7 hr. per day. 1205 aphides were trapped on 5 June, when the conditions were regarded as being ideal for flight. It was a particularly warm sunny day, the temperature remained above 70° F. well into the evening, and there was practically no wind. It was noticed that aphides were flying throughout the afternoon, and large numbers were on the wing between 5 and 7 p.m. Excellent conditions for the production of alatae prevailed in the previous weeks, and conditions were also favourable for flight later in the year, particularly at the beginning of August, but comparatively few aphides were trapped; this is believed to be due in part to the absence of large numbers of alatae in the field.

Factors influencing wing formation

It is generally accepted that starvation and warm dry periods, which produce wilting in plants, are factors which tend to accelerate the formation of wing buds in aphides. It has been shown experimentally by Schaefer (1938) that in the case of *Acyrthosiphon onobrychidis* Kalt. the number of alatae produced is inversely proportional to the ability of the plant to support its population; starvation on smaller plants produced more alatae.

Temperature is also an important factor in the tendency to produce wing buds. Ackerman (1926), working with *Rhopalosiphum prunifoliae* Fitch, showed that wing buds were more likely to be produced at 57·2–60·8° F. and at 75·2–78·8° F. than at 53 and 64·4–68° F. Wadley (1931), working with *Toxoptera graminum* Rond., concluded that the maximum number of winged forms was produced at 59° F.

The light factor is also important, Shull (1929), working with *Macrosiphum gei* Koch, states that "when parents were kept in continuous light or darkness, they produced exclusively wingless nymphs". "Winged nymphs were produced when parents were kept under alternating darkness and light." It is also possible that alternation of sunny and dull days, or sunshine and cloud, may affect wing bud formation.

Under the conditions usually obtaining in May, an aphis nymph would moult about every fourth day, so that a month prior to the production of alatae safely covers the period of wing production. An examination of the weather conditions for a month prior to large flights, or for the month of May in years when very few aphides were trapped, revealed that,

(i) In the good years, the fluctuations in daily temperature were greater than in the poor years: the figures given below are the average differences in daily temperatures:

Good years: 1935, 12·6° F.; 1936, 12·1° F.; 1939, 13·4° F.
Poor years: 1937, 10·9° F.; 1938, 11·1° F.

(ii) The good years were more sunny than the poor years, and had larger numbers of dry days:

Good years: (*a*) 1935: 17 dry and sunny days averaging 7·4 hr. sunshine,
(*b*) 1936: 16 dry and sunny days averaging 6·3 hr. sunshine,
(*c*) 1939: 23 dry and 22 sunny days averaging 6·7 hr. sunshine.

Poor years: (*a*) 1937: 9 dry and 5 sunny days averaging 4·5 hr. sunshine,
(*b*) 1938: 8 dry and sunny days averaging 4·7 hr. sunshine.

(iii) Correlated with the above, the relative humidities were high in the poor years, and lower in the good years; averages are given:

Good years: 1935, 77·4,
1936, 80·5,
1939, 79·8.

Poor years: 1937, 82·6,
1938, 89·1.

From these figures it seems that the most important factor tending to produce winged forms in Aphididae in general is the amount of sunshine during May. A comparatively sunny May producing large fluctuations in temperature and low relative humidities appears to accelerate wing formation. It is not suggested that all aphides react similarly to meteoro-

logical conditions; it is indeed very unlikely that this is so because of the sudden sporadic appearance of some species in very large numbers.

Incidence in the trap of particular species

(*a*) *Aphides trapped in comparatively uniform numbers in all good years*

Table 1 shows that the majority of species fall in this category. Typical examples of this group are *Aphis sambuci*, *Cavariella pastinaceae*, *Nasonovia ribicola*, *Metopolophium dirhodum* and *Macrosiphum rubiellum*. Of these, only *Cavariella pastinaceae* was trapped in comparatively large numbers, and it is probable that conditions for flight of this species compare closely with the conditions accepted as being good for the flight of aphides in general. The species which were trapped in small numbers in North Wales would probably be trapped in large numbers where the food plants are abundant, and the conditions under which those species fly in the field could be observed with more accuracy in such places.

(*b*) *Aphides trapped in varying numbers in different years*

This group includes the aphides *Brachycaudus helichrysi*, *Hyalopterus arundinis*, *Cavariella aegopodii* and *Cavariella unbellatarum*.

Brachycaudus helichrysi (Table 1, no. 9) was trapped over a long period in all years. The largest numbers were recorded in 1935 (305) and 1939 (347). Although the largest numbers were recorded at temperatures above 70° F., wind velocities below 5 m.p.h., and relative humidities below 90, this aphis was also trapped in considerable numbers under more adverse conditions. Sixteen were trapped at wind velocities above 5 m.p.h., 95 were trapped at temperatures below 60° F., and 95 were trapped at relative humidities above 90.

Cavariella aegopodii (Table 1, no. 32) was trapped in large numbers in 1939, when 570 were recorded, 95 were trapped in 1936, and 31 in 1935. The great majority flew in conditions about or better than the arbitrary standard suggested for aphis flight in general, but a few were trapped under more adverse conditions. Three were trapped at temperatures below 60° F. and 25 at relative humidities above 90.

Hyalopterus arundinis (Table 1, no. 12) was most prominent in 1935, when 146 were trapped; the numbers for all the other years were very low. The great majority were trapped under conditions better than the arbitrary standard.

Cavariella umbellatarum (Table 1, no. 35) was also trapped in large numbers in 1935 only. No specimens of this species were trapped at wind velocities above 5 m.p.h.

(*c*) *Aphides appearing sporadically in large numbers*

This group includes the aphides *Hyalopteroides dactylidis* and *Vacuna dryophila* (Table 1, nos. 31 and 69). Both these aphides appeared in very large numbers in 1939; only one specimen of the latter and none of the former was trapped in the previous years. The host plant of *Hyalopteroides dactylidis* is *Dactylis glomerata*, which is a very strong-growing and deep-rooted grass; this probably explains the trapping of alatae in 1939 only when drought conditions prevailed in May and June. It is unlikely that the drought conditions affected *Quercus* sp., which is the host of *Vacuna dryophila*, and it is possible that large numbers of alatae were produced by the direct effect of the meteorological conditions upon the aphis itself.

Summary

1. The Williams & Milne insect trap, as modified by Davies for use with water power, has been in operation in North Wales for five consecutive summers. The aphides trapped have been identified, and their incidence, as correlated with meteorological conditions, has been studied.

2. Seventy-six species are recorded, and the more important synonyms of some species of economic importance are tabulated.

3. It was found that under field conditions large flights of aphides took place when (*a*) the wind was from the north-east, (*b*) the wind velocity was very low (generally 0–2 m.p.h.), (*c*) the maximum temperature was above 75° F., and (*d*) the relative humidity was below 80. The most important factor limiting flight appears to be wind velocity; very few aphides were trapped when the wind velocity rose above 5 m.p.h.

4. In North Wales, the years 1935, 1936 and 1939 were good years for aphis migration, whereas in 1937 and 1938 very few aphides were trapped.

5. The factors which appear to stimulate the production of large numbers of alatae appear to be (*a*) large fluctuations in daily temperature, (*b*) a large percentage of dry, sunny days tending towards drought conditions, and (*c*) correlated with (*a*) and (*b*) low relative humidities. Certain aphides however, were trapped in large numbers only in 1939 when there was a long drought period. The effect of such a period on host plants is probably a very important factor governing the production of alatae.

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FACTORS INFLUENCING THE ABUNDANCE AND SIZE OF *PSYCHODA* SPECIES IN SEWAGE BACTERIA BEDS

By W. H. GOLIGHTLY, PH.D.

The Zoology Department, the University of Leeds

(With 1 Text-fig.)

THIS work extends studies on the fauna of bacteria beds of sewage works in which it was shown that interspecific competition for a limited food supply leads to a reduction in the numbers of the various flies breeding in the beds (Lloyd, 1937; Lloyd *et al.* 1940). Routine observations at one station with a moderately varied fauna have been paralleled with exactly similar records at another where the fauna is so restricted that the competition may be considered to be practically intraspecific. The observations have been continuous from October 1937 to April 1939 and have included the study and assessment of the fly ouput together with observations on the physical environment such as temperature, acidity and the nature of the medium. Further, the distribution of the larvae and pupae has been studied. As a result it has been possible to throw light upon certain aspects of competition between these flies, including the relation of size to temperature and crowding, and the significance of inter- and intraspecific competition.

MATERIAL AND METHODS

The stations where these observations were carried out were Huddersfield (Cooper Bridge) and Knostrop (Leeds).

Bacteria beds

The beds at Huddersfield are circular, 200 ft. in diameter and raised 7 ft. above the ground level. They are open-sided and the medium is of clinker. The sewage is distributed in jets by means of four arms which revolve quickly taking only 2–3 min. to complete a revolution. The cavities in the medium afford protection for all stages of the flies. Two beds were used for study, one (no. 1) with surface clinker consisting of pieces 1–2 in. in diameter and the other (no. 2) of 4 in. pieces.

At Knostrop the beds are rectangular and sunk to the ground level. The medium is of water-worn gravel, covered during most of the year with a growth of the blue-green alga, *Phormidium* (Reynoldson, 1939). The sewage is delivered in sheets, the distributing arms travelling slowly, taking ¼ hr. to traverse the length of the bed.

Definite resting periods are not the rule at either place. At Huddersfield each machine is stopped daily at any time for periods totalling 12–16 hr. (not continuously). The length of the rest depends chiefly on the amount of sewage to be treated, the rests being shorter or even absent during periods of heavy rainfall and longer during drought. Hence, although the surface of the bed may dry during these stoppages, there is no serious effect upon the fauna as the rest is never prolonged and only the top layer of 2–3 in. is affected. Bed temperatures were recorded at both places, and the Knostrop beds were found to be slightly cooler on the monthly mean (Table 9).

Character of sewage and treatment

The character of the sewage at Huddersfield is dominated by the waste products of the dye works in the district and consequently is acid in reaction. The crude sewage at times has a *p*H 3–4. It is neutralized by means of lime so that the water reaching the beds averages *p*H 6–7. Lime is not added on Sundays, and hence very acid sewage is delivered on to the beds for several hours at that time. During holiday, when the dye works are closed, the sewage becomes alkaline. At Knostrop the sewage is not dominated by any one industrial waste and is approximately neutral.

At the height of summer the output of *Psychoda alternata* from the beds at Huddersfield is excessive and an insecticide is used to control the fly output (Scouller & Goldthorpe, 1932). No similar drastic chemical treatment of the sewage occurs at Knostrop. During the period of the observations recorded here, treatment of the sewage with the insecticide started in mid-April, was continued daily for a month, and thereafter when the fly output was again showing indications of becoming excessive. It was discontinued in September even though the number of flies was relatively high, as experience has shown that drastic reduction at this time might seriously jeopardize the chances of some life surviving the winter and hence interfere with the working efficiency of the beds. However, this treatment may have an enduring influence upon the composition of the bed fauna. The details of the treatment are as follows:

Creosote, soda-ash and soap, thoroughly mixed for $\frac{1}{2}$ hr., is put on to the beds at the rate of one barrel per day. This quantity, mixed with the great bulk of the sewage as it passes on to the beds, gives a dilute concentration and is exhausted in 10 min. The bed is then rested for 1 hr. so that the creosote is held by the medium for a short time. It has been noticed on frequent visits to the beds that the smell of creosote persists for some time after the last dosage, but in spite of this the quick recovery of the fly output is remarkable (see p. 409).

Flora and fauna

No thick algal growth comparable to that at Knostrop occurs on the beds at Huddersfield but a brownish grey film is found (chiefly in winter when the fly numbers are low) consisting of fungi and other vegetable matter. Nematodes abound in this film.

Psychoda alternata Say., *P. severini* Tonn. and *Spathiophora hydromyzina* Fln. are common to the beds of both places. Knostrop has in addition the chironomids *Metriocnemus longitarsus* Goet., *M. hirticollis* Staeg. and *Spaniotoma minima* Mg., while Huddersfield has a few *Psychoda cinerea* Banks, lacking from the Knostrop beds. The bed fauna in general is more fully discussed by Lloyd *et al.* (1940). Some condition in the Huddersfield beds is adverse to the chironomids, since only two individual *Metriocnemus* spp. and three *Spaniotoma minima* have been trapped in 18 months. The fact that the tank effluent is slightly acid and on Sundays often falls to *p*H 3 may account for the absence of *Metriocnemus*, as Reynoldson (unpub.) has observed that an acid medium is detrimental to the formation of an egg ribbon in this fly.

Method of trapping

The general method adopted was that of Lloyd *et al.* (1940), records being collected from a glass jar over a perforated wooden tray 1 ft. square, rough estimates of abundance taken from accumulations underneath the tray and counts from tins ($4 \times 2{\cdot}5$ in.) sunk into the surface of the beds. Several records are required because *Psychoda alternata* is not strongly attracted to daylight. Thus three distinct assessments of *Psychoda alternata* and one of *P. severini* were obtained, the numbers of the latter insect under the trays not being estimated as they were swamped by the large accumulation of *P. alternata* and few were recorded in the tins.

Distribution of larvae

To ascertain the distribution of larvae and other life in the beds food bags were placed at various depths. These consisted of muslin bags in each of which was placed 15 g. of scalded alga from Knostrop, experience having proved this to be a convenient bait. The alga was examined before use to remove all macroscopic life. The bags were lowered into covered pipes at depths of 4, 12 and 30 in. They were changed weekly and their contents were treated by Reynoldson's method (1939).

Measurement of flies

The body proportions of these flies remain constant so that the wing length is a reliable index of size. The distance from the semichitinous pad at the base of the costa to the tip was taken as an indicator (del Rosario, 1936). All subsequent mention of fly size refers to wing length. Fifty males and fifty females were measured from each bed each week whenever possible. The differences between the arithmetic means were regarded as significant if they were greater than twice the standard errors for the values concerned.

Up to the end of February 1938, samples from a mixed lot of flies from beds nos. 1 and 2 were measured but after this they were treated separately in order to detect any relationship between density and fly size.

Observations and results

Fly trappings. The abundance and seasonal incidence of the flies as evidenced by the trappings is shown in Table 1. Until the normal working of the beds is interfered with by the creosote treatment, the *P. alternata* output of bed no. 1 exceeded appreciably that of bed no. 2. When treatment started however the preponderance was reversed and the output of bed no. 2 slightly exceeded that of no. 1. The treatment effected a 90% reduction in the first week on bed no. 1 as opposed to an initial decrease of 70% on bed no. 2. The excess of no. 2's output was much greater than the figures indicate during the greater part of the treatment however but is masked by a large outburst from no. 1 during September when treatment took place on only four days—5, 11, 23 and 24 September.

During the period of no treatment, viz. October 1937–11 April 1938 and 25 September 1938–April 1939, there were 362 assessments of abundance of *P. alternata* from each bed (206 from jars, 50 from tins and 106 rough estimates from the trays). During treatment (12 April—24 September 1939) there were 154 assessments from each bed (86 from jars, 26 from tins and 42 from the trays). An analysis of these assessments is given below:

	Total no. of assessments	No. of assessments in which no. 1 in excess	No. in which equal	No. of assessments in which no. 2 in excess
No treatment	362	248 (68·5%)	33 (9·1%)	81 (22·3%)
Treatment	154	49 (31·8%)	2 (1·3%)	103 (66·9%)

It is probable that the closer aggregation of the clinker on no. 1 retains the creosote fumes for a longer period than the more open coarse bed and hence causes a greater reduction in numbers.

Table 1. *Seasonal incidence and abundance of* Psychoda spp. *at Huddersfield*

	P. alternata (bed no. 1)			*P. alternata* (bed no. 2)			*P. severini* (jars only)	
Month	Jars	Tins	Trays	Jars	Tins	Trays	Bed no. 1	Bed no. 2
Oct. 1937	1145	408	19000	637	224	16000	1	0
Nov.	565	724	23000	610	432	16000	2	2
Dec.	203	529	10300	194	141	2350	1	2
Jan. 1938	266	1245	4700	173	129	1000	1	0
Feb.	196	496	4700	217	134	1700	2	5
Mar.	1694	922	10700	735	321	4100	55	30
1–12 Apr.	2025	807	36400	1121	143	21200	200	93
	6094	5131	108800	3687	1524	62350	262	132
13–30 Apr.	696	67	800	871	115	2000	12	34
May	633	110	950	1490	332	2650	294	282
June	939	59	1110	2507	376	2650	119	114
July	1135	130	4700	2191	362	6600	85	282
Aug.	626	445	3490	880	240	2750	41	96
1–24 Sept.	2500	3329	20500	1061	1222	7100	5	9
	6529	4140	31550	9000	2647	23750	556	817
25–30 Sept.	722	440	1800	707	677	2600	17	16
Oct.	1058	4393	10200	1310	1530	6800	7	20
Nov.	1800	2544	12000	1189	3104	9100	0	1
Dec.	462	419	3500	171	546	1450	34	39
Jan. 1939	68	196	2730	103	61	1170	13	46
Feb.	189	177	1340	119	56	570	22	159
Mar.	282	73	2260	302	45	2160	99	478
	4581	8242	33830	3901	6019	23850	192	759

Treatment period 13 Apr.–24 Sept. inclusive.

The rate of recovery after treatment is rapid. For example, owing to rather heavy treatment during August 1938 (the beds were treated on 14 days) the weekly total catch for the first week of September for the two beds was only 335 *P. alternata*. The catch from the two beds however produced 1744 in the second week and regularly exceeded 1000 per week throughout the rest of the month.

This suggests that the pupal and imaginal stages are more susceptible to treatment than are the egg and larval stages. If the eggs and larvae were greatly reduced, time enough for a complete life cycle must necessarily elapse before the fly numbers could be replenished. Even at the summer bed temperatures this would take 3–4 weeks and it therefore seems that the eggs and larvae are not so seriously affected as the pupae and adults.

The size of the flies is discussed below (see p. 415).

Laboratory observations

Laboratory investigations were designed to examine two aspects of size in *Psychoda*, (1) the factors influencing size during growth, and (2) the influence of parent size on the succeeding generation. Information on the effect of graded temperatures on viability was also collected. These will be briefly discussed.

Parent flies were measured, the female always and the male when known. Ovipositions were made on small wisps of wool in which the egg batches could be counted, the eggs measured and the hatching rate observed. The length of the elongated egg was the standard of size and ten formed a sufficient sample of the batch. In many cultures the whole batch was reared as a single family in a 4 oz. jar on an excess of food (scalded aerated *Phormidium*) on a pad of cotton wool (Lloyd, 1935). In other cultures numbers were restricted to ten larvae and reared on a constant sufficient food supply (0·5 g.) in tubes kept at carefully graded temperatures. In others larvae in increasing multiples of ten were reared on an adequate supply of food (1·5 g.). In another series the food supply was kept inadequate for the larger groups. Most of these tests were made with the three species of *Psychoda* and in general they were performed for each at three temperature grades, approximately for *P. alternata* 27, 17–19 and 10–12° C.; for *P. severini* 20, 15–18 and 11–14° C.; for *P. cinerea* 17–20, 13–15 and 9–12° C., i.e. in an incubator, a warm room and a cool cellar respectively. Records of survival to maturity, time of development, sex proportions and dimensions of the offspring (wing length) were made. In nearly every case the male offspring had approximately the same relative dimensions as female offspring so only the latter will be recorded.

Table 2 shows the effects of temperature during development on size both for single families, where there is some influence from a variable degree of crowding, and for limited numbers of larvae (10) in culture. That the size of insects tends to vary inversely with the temperature during growth is well known (Uvarov, 1931), but it is important to discover the degree to which this influence is felt in *Psychoda* in order to understand the size of the wild flies.

TABLE 2. *Effect of temperature on size*

	P. alternata		*P. severini*		*P. cinerea*
	Natural families	Limited cultures	Natural families	Limited cultures	Natural families
No. of tests	... 58	44	45	24	36
Temp. ° C.					
27	1·72 ± 0·022	—	—	—	—
20	—	—	1·88 ± 0·012	—	—
18	2·00 ± 0·012	2·05 ± 0·007	—	—	1·95 ± 0·036
17	—	—	2·06 ± 0·049	1·93 ± 0·014	—
14	—	2·11 ± 0·027	—	2·16 ± 0·023	2·17 ± 0·038
13	—	2·19 ± 0·040	2·13 ± 0·050	—	—
11	2·16 ± 0·042	2·24 ± 0·019	—	2·36 ± 0·019	2·18 ± 0·050
9	—	2·28 ± 0·039	—	—	—

It is natural that shortage of food should affect size and surviving numbers and effects were evident in the experiments. This factor might also prolong development and affect the sex proportions but the series of tests were insufficient to discover any such influences. Crowding in culture when food is abundant is also well known to influence size and the reasons for this have been much discussed (Allee, 1931; Bodenheimer, 1938); as these results conform to the general findings they need not be detailed. It may, however, be pointed out that the effect of crowding in the whole families was evident since there was in all cases a negative correlation between family size and offspring size and the correlation is significant in six of the temperature groups out of nine shown in Table 3.

TABLE 3. *Coefficient of correlation between family numbers and size of individual in whole family cultures of* Psychoda *with excess food and in* 4 *oz. jars*

	P. alternata		*P. severini*		*P. cinerea*	
Temp. ° C.	Cultures	Correlation coefficient	Cultures	Correlation coefficient	Cultures	Correlation coefficient
27	15	−0·50	—	—	—	—
20	—	—	12	−0·37	—	—
18	24	−0·79	—	—	11	−0·33
17	—	—	8	−0·43	—	—
14	—	—	—	—	14	−0·60
13	—	—	25	−0·63	—	—
11	19	−0·74	—	—	11	−0·79

A possible relationship between parent size and offspring size should also be considered, though such would hardly be expected in a community of insects mainly inbreeding (Lloyd, 1935). The coefficients of correlation in this regard were calculated and for the same cultures grouped under temperature grades as shown in Table 3 are: *P. alternata* +0·07, +0·01 and −0·43; *P. severini* −0·74 (at 20° C. a harmful temperature), −0·14 and −0·16; *P. cinerea* +0·05, −0·01 and −0·40. There is thus no evidence of correlation. Larger females, as will be shown, lay more eggs than small ones and if all the offspring survived a variable degree of crowding in culture would give a false negative correlation between parent size and offspring size but probably variable mortality has obviated this influence.

In considering the influence of parent size on the number and size of the eggs the temperature grouping is disregarded. As *Psychoda* feeds little in the adult stage, is normally fertilized early and, except at low temperatures, matures the eggs and oviposits all of them in the course of a day or two, sometimes in a single operation, it is likely that size and nutrition of the larvae determine the number and the size of the eggs. Since temperature during growth mainly determines the size of the fly it would be impossible to condition the insects of varied size properly for oviposition at graded temperatures. In considering these aspects therefore the flies tested are sorted into three arbitrary size groups of large, medium and small flies, the grade being 0·4 mm. wing length. *P. alternata* available ranged from 2·8 to 1·4 mm., *P. severini* from 2·64 to 1·72 mm., only two flies falling in the large group, and *P. cinerea* from 2·60 to 1·88 mm., only three flies falling in the small group. The average number of eggs laid and the size of these are shown in Table 4 and it is evident that the number of eggs laid is directly related to size and that larger flies also tend to lay larger eggs, the group of small *P. cinerea*, which included only three flies of such a size that they just missed inclusion in the medium group, being discounted.

TABLE 4. *Relationship between size of parent and mean number and size of eggs. Measurements in mm.*

	P. alternata		*P. severini*		*P. cinerea*	
	No. of eggs	Size of eggs	No. of eggs	Size of eggs	No. of eggs	Size of eggs
Total no. tested ...	39	35	33	34	30	29
Parent size grade						
2·80–2·40	158·4 ± 9·82	0·338 ± 0·007	181 (2 flies)	0·320 (2 flies)	219·4 ± 21·84	0·251 ± 0·005
2·36–2·00	135·8 ± 12·92	0·335 ± 0·006	98·6 ± 6·78	0·285 ± 0·005	145·1 ± 7·65	0·241 ± 0·004
1·96–1·40	79·7 ± 5·58	0·314 ± 0·005	76·5 ± 9·29	0·269 ± 0·011	142 (3 flies)	0·247 (3 flies)

Effect of temperature upon viability

One other set of factors must be considered before a comprehensive discussion can be made upon the data obtained from the field observations. This concerns the viability of the eggs and larvae of the three species under different temperature conditions.

In Table 5 the results are expressed for the above temperature ranges for each species. The percentage egg viability, the percentage larval viability (a few pupal deaths are probably included in this estimate) and lastly the percentage of eggs which complete their development and ultimately produce flies are recorded. These results are calculated from the data obtained from all cultures carried through at each temperature and therefore represent the

TABLE 5. *Influence of temperature on viability of eggs and larvae*

P. alternata			
Temp. ° C. ...	27	17–19	10–12
% egg viability	75·4 ± 4·337	83·7 ± 4·944	89·5 ± 2·135
% larval viability	57·8 ± 4·039	68·3 ± 6·55	38·7 ± 5·493
% imagines produced/ no. of eggs	51·1 ± 4·673	53·5 ± 2·369	38·5 ± 5·493
P. severini			
Temp. ° C. ...	17–20	11–14	
% egg viability	42·8 ± 9·391	87·5 ± 3·468	
% larval viability	53·6 ± 5·975	72·9 ± 3·017	
% imagines produced/ no. of eggs	18·0 ± 3·977	85·5 ± 5·504	
P. cinerea			
Temp. ° C. ...	17–20	13–15	9–12
% egg viability	73·1 ± 5·806	70·8 ± 9·451	51·7 ± 9·803
% larval viability	47·4 ± 4·578	36·5 ± 4·421	43·0 ± 7·747
% imagines produced/ no. of eggs	36·8 ± 7·712	30·8 ± 6·478	22·8 ± 7·426

average of a large number of observations. These results indicate several points which will be relevant in the discussion. The most import are:

(1) That egg viability is fairly high for all species at all temperatures employed with the exception of *P. severini* at 17–20° C. and *P. cinerea* at 9–12° C.

(2) That the percentage of eggs which finally produce imagines is fairly high from 17° C upwards for *P. alternata* and low for *P. severini* whilst the reverse is true at lower temperatures 10–13° C. *P. cinerea* has a wide range in this respect, the results being little different from 13 to 20° C. with a decrease below 13° C.

(3) That larval viability is favoured by low temperatures for *P. severini* and higher ones for *P. alternata*, whilst *P. cinerea* is little affected by temperature in this range.

These results, which cover bed temperature range during most of the year, confirm the conclusions of Lloyd (1937) that in the colder months temperature conditions favour *P. severini*, whilst *P. alternata* has a distinct advantage in summer and early autumn. Although the temperatures are favourable for *P. cinerea* during most of the year, the survival values of its various stages in the laboratory are lower than those of the other two species and the weakness indicated might account for its scarcity at Huddersfield, its numbers not being sufficiently high to compete successfully with *P. alternata* and *P. severini*. The eggs of *P. cinerea* are on the average smaller than those of the other two species (see p. 411) and hence the first instar larvae are correspondingly smaller. This may be an important factor in competition and account for its relative scarcity in nature.

Discussion

The field observations show that abundance and temperature are strongly related and that there is a large variation in size.

The experimental studies were undertaken to investigate the cause of this size variation. They indicate that temperature and size are inversely proportional. The importance of crowding and abundance upon size was also investigated and linked with temperature. It was found that size and abundance were conditioned by temperature whilst abundance and size were also interrelated. In addition the supply of food must play a vital part, influencing both size and abundance. The analysis of the interrelationships of these factors has clarified considerably our knowledge and information upon the competition existing in the bacteria beds.

Previous studies of the sewage bed fauna (Lloyd, 1935, 1937; Lloyd *et al.* 1940) have shown that chironomids exert an important control on the abundance of *Psychoda*. The present study was undertaken to observe the behaviour of *Psychoda* in beds that maintain no chironomids. The drastic reduction of the *Psychoda* at Huddersfield by treatment during summer renders it useless to extend the comparison to the completed year and figures are given for two periods, viz. October 1937—12 April 1938 and 25 September 1938–April 1939, twelve and a half months in all, but not including the time when the Psychodas have their greatest potential abundance. During the periods in question trapping was on exactly parallel lines at two positions on each of two beds at Huddersfield and at three positions on one bed at Knostrop. Table 6 gives the total catches for this period on 1 sq. ft. of bed, except the second estimate of *P. alternata* which represents the numbers caught in a dark tin.

Table 6. *Total catches on 1 sq. ft. of bed*

Locality ...	H 1	H 2	K 1	K 2	K 3
P. alternata: jars	4972	3440	341	367	230
tins	6367	3433	361	486	673
P. severini	227	445	245	544	146
Spaniotoma minima	0	0	12089	13647	17078
Metriocnemus spp.	0	0	1939	3096	2771

P. alternata is some ten times as abundant at Huddersfield but there is no appreciable difference in the *P. severini* output. The deficiency in the *P. alternata* at Knostrop is compensated by the heavy output of chironomids. The figures for the latter and *P. severini*

are nearer the true output than those for *P. alternata* (see p. 407) which are simply relative to the actual emergence and the activity of the imagines induced by equable external conditions. These are approximately the same at both places.

Comparison of the Huddersfield food bag data with that obtained by Lloyd *et al.* (1940) at Knostrop further helps towards solving the problem of the difference of the *Psychoda* output at the two places. The trappings were not strictly parallel, continuing for 44 weeks at Knostrop (June 1937–April 1938) and for 14 weeks at Huddersfield (17 January–4 April and 7–28 June 1939). The comparison however is instructive.

The results are compared in Table 7 which indicates the total number of larvae trapped and the weekly average of each species at 12 and 30 in. for Knostrop and at 4, 12 and 30 in. for Huddersfield.

TABLE 7. *Comparative numbers of larvae trapped at Huddersfield and Knostrop*

	Huddersfield (14 weeks)			Knostrop (44 weeks)		
in.	*P. alternata*	*P. severini*	*Metriocnemus*	*P. alternata*	*P. severini*	*Metriocnemus*
4	3196, av. 355	651, av. 72	—		No record	
12	689, av. 45	899, av. 64	—	484, av. 11	—	5647, av. 128
30	984, av. 70	1100, av. 80	—	3007, av. 68	—	1077, av. 25 (932 in one catch)

Fair (1934) states that although *Psychoda* larvae are found throughout the depths of the bacteria beds, they are most abundant in the top 3–12 in. This is confirmed by the Huddersfield results. The Knostrop record, however, shows the reverse—a greater abundance of *P. alternata* larvae at deeper levels down to 30 in. at least. This is due presumably to the occupation of the topmost zone by *Metriocnemus* at Knostrop as it will be seen that the numbers of *Psychoda* spp. and *Metriocnemus* are in inverse proportion at 12 and 30 in. Here then we have evidence of inter-specific competition with its resulting decrease in the numbers of *Psychoda* due to *Metriocnemus* inhabiting that part of the beds which is normally chosen by and presumably most favourable to *Psychoda*.

The reason for the absence of chironomids at Huddersfield is not clear but may be explained as due to the operation of either of two factors: (1) The acid nature of the sewage might be adverse to the eggs or skin breathing larvae. Though the acidity is normally neutralized by lime, sewage of *p*H 3 reaches the beds for several hours on Sundays, and (2) The creosote treatment might prevent their establishment.

The presence of *P. alternata* causes annoyance at sewage works and the vast number arising from the Huddersfield beds renders some reduction imperative and the creosote treatment is calculated to reduce the excessive breeding whilst leaving enough larvae to keep the beds open. It is probable that all stages are affected to some degree but it is clear from the trapping data that the mortality is mainly caused in the adult and pupal stages (see p. 409). This treatment upsets the normal seasonal incidence curve as obtained at Knostrop and renders comparison in this respect impossible. Besides affecting conditions at the time of treatment it may have an indirect effect upon the fauna, its numbers and the size of individuals at other times of the year. Hence the possible effects of treatment must be borne in mind when comparisons are made.

Three factors during growth bring about a reduction in the size of insects: rising temperature, shortage of food, and crowding, either because the bulk of excretory product in relation

to the food available sets up an intoxication, or because the induced restlessness due to frequent contact increases the rate of metabolism in the larvae (Lynn & Edelman, 1936). The effects of all three factors have been investigated by culture methods in the present study for *P. alternata*, *P. severini* and in part for *P. cinerea*. It must now be considered to what extent their influence can be demonstrated in the beds.

First, are the three factors present in the beds?

(1) *Temperature*. There is a fairly wide temperature range according to the monthly means from 6 to 18° C.

(2) *Shortage of food*. The basic food supply, the amount of solids in suspension and solution in the sewage reaching the beds, does not vary in any important degree through the year. The actual amount of food available however may vary as the nutritive quality of the basic supply is fixed and changed by varying physical agencies. At Knostrop for instance the growth of *Phormidium* on the surface is an important food supply and its formation is most active during the longer days (Reynoldson, 1939). Nothing definite can be said about this factor at Huddersfield where there is no surface growth or deposit except during the colder months when the fly numbers are low. Shortage of food has been demonstrated at Knostrop and occurs especially in the late spring when it becomes so acute that the larvae and worms migrate downwards in the bed and even pass through the floor in great numbers (Reynoldson, 1939; Lloyd *et al.* 1940). This shortage is associated with the spring off-loading which is a sloughing of the surface and deep zooglaeal film on the medium and is a well-known phenomenon in bacteria beds. It is caused by the increasing activity of the inhabitants as the temperature rises, combined with a general looseness of the film due to the events of winter. Local shortage of food can also be deduced by the observation of larvae searching for food on apparently clean medium.

(3) *Crowding*. This is very evident at times. At Huddersfield the numbers of flies are so great prior to treatment, that considering the number of eggs laid and the hatching and survival rates (see p. 411) there must inevitably be crowding. Evidence of intense crowding at Knostrop has been given in previous work (Lloyd *et al.* 1940).

In studying the variation of size of the flies in the bed, it is not practicable to separate the effect of food shortage from that of crowding because the latter almost inevitably entails at any rate a local shortage of food. There is even some difficulty in assessing separately the effects of temperature and the other combined factors, because as temperature rises the rate of breeding increases, so that all the factors tend to be intensified together.

The value of the record of flies emerging from the beds as an indication of the density of the larval population should be considered. The first consideration is that the *Psychoda* need not leave the bed and though *P. severini* is active in cold weather and enters the trapping jars freely in winter, *P. alternata* is very sluggish under these conditions and in mid-winter, the numbers observed at the surface in any of the traps may not give therefore a true indication of the number of imagines actually present and hence of the number of larvae in the bed. However, the Knostrop records seem to indicate that the flies trapped at the surface do bear a reasonable ratio to the density of the larvae in the bed. The larvae were trapped and recorded through about a year and the coefficient of correlation between trapped *Psychoda* larvae (3rd instar) and the emerging fly is +0·70, a figure which indicates a fair agreement. For the same period the coefficient of correlation between the trapped *Metriocnemus longitarsus* larvae and the emerging fly was +0·74. On the other hand evidence was found at Knostrop

that one part of the bed may become so heavily populated with larvae of *M. longitarsus* that the output of imagines may be reduced and a false idea of the larval density may be obtained by a record of the emerging flies alone (Lloyd *et al.* 1940). The same sort of thing can be illustrated for *Psychoda*. The first peak of *P. alternata* imagines at Knostrop occurred in 1938 on 17 March with a maximum tray record of 1500 (average of three positions) and the next peak on 22 April with an average tray record of 7000 (the highest of the year). The tray records were then generally above 1000 until the end of May but thereafter declined till in August they were of the order of 100. Now the April peak must have been followed by the heaviest oviposition of this fly for the year and therefore by the highest potential larval density. About this time however food becomes scarce correlative with increasing competition and increased larval mortality results from this and other factors in the competition, such as predacity and loosening of the growth. Therefore larval mortality from one cause and another reduces the fly output in relation to the true larval density in the middle of summer and the now well-known two peaked curve of incidence of *P. alternata* results. The same thing can be shown for *P. severini* but there is no autumn recovery for this insect at Knostrop. Similar phenomena could no doubt be illustrated at Huddersfield but for the treatment. Because of these factors it is not possible to deduce exactly the degree to which temperature rise on the one hand and the correlative increase in breeding activity, leading to crowding, on the other, influence the insect size.

The most convenient approach to the effect of temperature on size seems to be an artificial sorting of the data, referring each sample of flies measured to the mean temperature of the bed during their month of emergence. This method is artificial to the extent that, within the groups, size will in some cases have been influenced by the temperature of the preceding months, and this protracted influence will be greater in the winter months when the rate of development is slower. It is known that the influence of temperature on size is mainly effective in the last larval instar, during which most growth takes place. In the case of *P. alternata* the pupal periods are approximately: at 15° C., 3–4 days; at 10° C., 10 days; at 7° C. (about the lowest in the beds), 15 days. Thus, this defect in the method of presentation is not great above 10° C. but is rather serious towards the bottom of the scale. The case with *P. severini* is similar.

The data are sorted in this way for *P. alternata* in Table 8 and Fig. 1, a temperature gradient of 2° C. being employed, thus giving six groups of measurements for the species. For each item is given:

(1) The average wing length with standard error for the sample.
(2) The place of origin with an indication of bed or trapping site.
(3) The month of capture.
(4) The estimates of abundance.

The Huddersfield flies are segregated above and the Knostrop flies below as the abundance of *P. alternata* is in general so much in excess at the former place. In each of these place groups the samples are arranged in order of magnitude and in each an "*s*" prefixed to the measurement indicates that the sample is significantly smaller than the maximum size in that place group. In Fig. 1 place of origin is ignored and all the samples are indicated under their appropriate temperature gradient. For each temperature gradient it may be taken that the highest value is the closest approximation found to the largest size the flies could attain in the beds with adequate food and in uncrowded conditions and that those groups of flies

whose dimensions are significantly smaller than this found conditions—food supply or density—to a varying degree adverse. For *P. alternata* the maximum size occurs at 8–9° C., the top group at 6–7° C. being significantly smaller than several groups in the 8–9° C. gradient. This may be due to the artificial nature of the grouping or it may be that very low temperatures are decidedly adverse to growth for this species as they are known to be adverse to larval viability (Lloyd, 1937). Taking the 8–9° C. maximum as a standard, the maxima in the succeeding columns fall by a difference of approximately 4% for each 2° C. rise of temperature. These factors are shown at the foot of Fig. 1 and a similar percentage range within each temperature gradient is also shown. The latter are 2–3 times as large as the

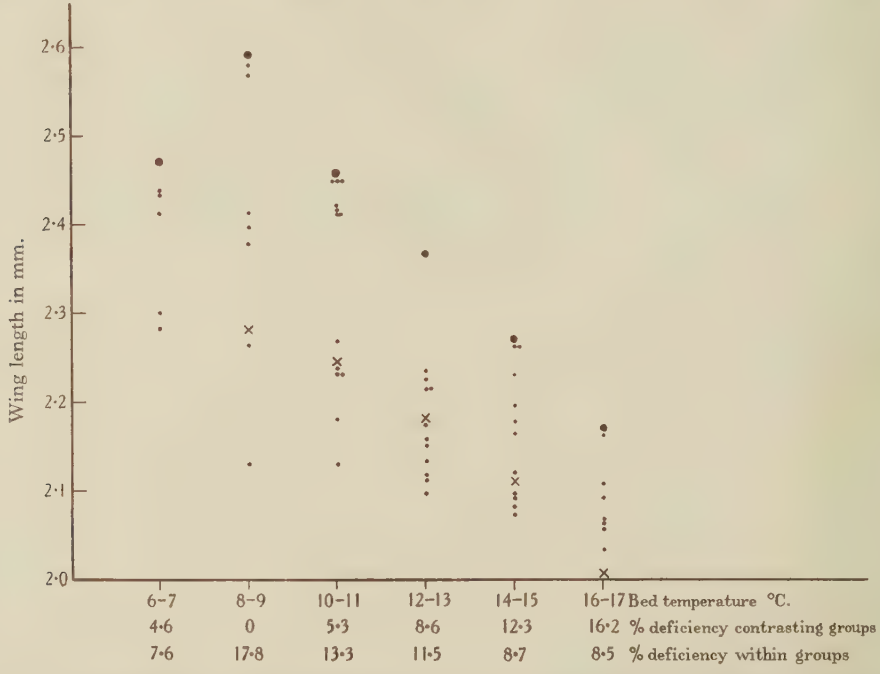

Fig. 1. *P. alternata* females showing wing length in relation to mean monthly temperature during month of emergence. The percentage deficiency from the largest for each difference of 2° C. and the greatest percentage deficiency in each temperature group are shown. The crosses indicate the size of the ♀ laboratory bred flies at the temperatures concerned.

difference between two temperature gradients and the conclusion is drawn that the effect of larval density is evident. *P. severini* has been examined in the same way with closely similar results. The maximum monthly size (2·53 mm.) occurred at Knostrop in January 1939 and falls in the 6–7° C. category. The smallest monthly sizes (1·91 and 1·77) occurred at Huddersfield in July and August and fall into the 16–17° C. group. Between these two extremes the fly size becomes steadily less as temperature rises and the difference for each 2° C. is again about 4%. Further details are not given as they would be largely a repetition of what has been said for *P. alternata*. It may be remarked that *Spaniotoma minima* and *Metriocnemus longitarsus* have been dealt with in the same manner and gave closely similar results (Golightly & Lloyd, 1939), i.e. if the data are arranged in diagram form sorted as in Fig. 1 the maximal points form practically a straight line with the absolute maxima at 8–9° C.

TABLE 8. P. alternata, *Huddersfield and Knostrop. Female wing lengths grouped under temperatures during months of emergence. Abundance in jars, tins and under trays is also shown*

Size	Site	Month	Jar	Tin	Tray
6 and 7° C.					
2·47 ± 0·017	H_1	Jan. 1939	34	196	272
2·43 ± 0·026	H_2	Jan. 1939	52	61	117
s 2·28 ± 0·012	H_2	Dec. 1938	85	546	242
2·47 ± 0·047	K_2	Jan. 1939	2	27	32
2·42 ± 0·035	K_2	Dec. 1938	2	33	800
s 2·33 ± 0·022	K_2	Feb. 1938	14	2	937
8 and 9° C.					
2·49 ± 0·014	H_2	Mar. 1939	151	45	270
s 2·41 ± 0·023	H_2	Feb. 1939	24	56	71
s 2·40 ± 0·016	H_1	Feb. 1938	98	496	588
s 2·38 ± 0·013	H_1	Feb. 1939	95	177	168
s 2·13 ± 0·015	H_1	Dec. 1938	231	419	583
2·59 ± 0·080	K	Mar. 1939	1	4	40
2·58 ± 0·022	K_1	Jan. 1938	8	1	533
2·57 ± 0·036	K	Dec. 1937	2	14	53
s 2·26 ± 0·041	K	Feb. 1939	1	2	20
10 and 11° C.					
2·45 ± 0·012	H_1	Mar. 1939	141	73	283
s 2·42 ± 0·007	H_2	Mar. 1938	368	321	410
s 2·27 ± 0·012	H_2	Apr. 1938	1121	286	5240
s 2·23 ± 0·014	H_2	Nov. 1938	595	3104	1140
2·45 ± 0·016	K_3	Mar. 1938	61	344	700
2·45 ± 0·019	K_3	Nov. 1938	17	12	71
2·45 ± 0·019	K_2	Nov. 1938	91	158	1300
2·42 ± 0·023	K_1	Nov. 1938	66	1	100
2·41 ± 0·018	K	Nov. 1937	9	0	365
2·41 ± 0·024	K_1	Mar. 1938	41	175	475
s 2·24 ± 0·022	K_2	Apr. 1938	247	397	2500
s 2·24 ± 0·015	K_2	Mar. 1938	84	0	1500
s 2·18 ± 0·022	K_3	Apr. 1938	268	347	4375
s 2·13 ± 0·028	K_1	Apr. 1938	142	793	2750
12 and 13° C.					
2·37 ± 0·015	H_1	Mar. 1938	847	9222	1070
s 2·23 ± 0·001	H_1	Oct. 1938	529	4393	1250
s 2·18 ± 0·025	H_2	May 1938	745	332	265
s 2·17 ± 0·013	H_1	Oct. 1938	65	1350	850
s 2·13 ± 0·028	H_2	Apr. 1938	2025	1614	9100
s 2·12 ± 0·015	H_1	Nov. 1938	900	2544	1200
2·23 ± 0·016	K_2	May 1938	150	162	1070
2·21 ± 0·022	K_1	Oct. 1938	52	16	152
2·21 ± 0·019	K_3	Oct. 1938	18	41	73
s 2·16 ± 0·020	K_2	Oct. 1938	58	133	235
s 2·15 ± 0·014	K_1	May 1938	207	184	1475
s 2·10 ± 0·015	K_3	May 1938	114	346	2025
14 and 15° C.					
2·27 ± 0·014	H_2	June 1938	1245	376	531
2·26 ± 0·019	H_1	June 1938	470	59	139
s 2·09 ± 0·015	H_1	May 1938	312	100	95
2·27 ± 0·016	K_1	Sept. 1938	102	230	400
s 2·23 ± 0·014	K_2	Sept. 1938	217	500	1060
s 2·19 ± 0·021	K_3	Sept. 1938	139	177	545
s 2·18 ± 0·019	K_2	July 1938	87	217	200
s 2·12 ± 0·024	K_1	June 1938	100	201	275
s 2·10 ± 0·036	K_1	July 1938	51	184	217
s 2·08 ± 0·029	K_3	July 1938	18	151	90
s 2·07 ± 0·036	K_3	June 1938	197	112	825

TABLE 8 (*continued*)

Size	Site	Month	Jar	Tin	Tray
16 and 17° C.					
2·17 ± 0·003	H_2	July 1938	1096	362	1100
2·16 ± 0·015	H_1	July 1938	567	130	783
s 2·09 ± 0·008	H_2	Aug. 1938	440	240	275
s 2·06 ± 0·011	H_1	Aug. 1938	313	445	349
s 2·06 ± 0·009	H_1	Sept. 1938	1610	3769	2788
s 2·04 ± 0·013	H_2	Sept. 1938	883	1899	1215
2·11 ± 0·025	K_3	Aug. 1938	167	109	82
2·06 ± 0·020	K_2	Aug. 1938	52	167	95
s 1·98 ± 0·027	K_1	Aug. 1938	42	36	87

There is little doubt that these curves do indicate the real relationships existing in the bed between the temperature and the size of the flies. The laboratory findings from breeding *P. alternata* with adequate food and in uncrowded conditions are also illustrated in Fig. 1, the wing length at each temperature gradient being indicated by a "×". The laboratory bred flies are smaller at each temperature gradient than the largest wild flies. The most probable explanation of this is that the food used in culture is in some way not quite so suitable as that obtained in the beds. It should be noted that the culture food is scalded to destroy extraneous organisms.

Table 8 for *P. alternata* shows that in most of the place groups there is a tendency for the smaller flies to be associated with larger populations as seen in the numbers emerging from the beds. However there are many exceptions and statistical analysis based on this artificial grouping is not possible. In the case of the Knostrop flies a dense population of one of the competing species may often be a determining factor but the data available are not yet sufficient for an analysis of this point. The Huddersfield flies afford a simpler case since *P. alternata* is so much the dominant insect there throughout the year. The data obtained from the months in which there was no treatment are considered, i.e. October 1937–12 April 1938 and 24 September–April 1939. This gives a limited temperature range of about 7·5° C. on the monthly mean temperature. In these months the correlation coefficients between temperature and size, based on thirty observations, are −0·658 for bed no. 1 and −0·561 for bed no. 2; and that between density and temperature is +0·561 for bed no. 1 and +0·647 for bed no. 2; and that between density and size is −0·232 for bed no. 1 and −0·373 for bed no. 2. All these coefficients are significant with the exception of the −0·232 value.

These coefficients have been examined by the system of partial correlation (Fisher, 1928) with the following results:

Bed no. 1:	Temp.—Size (abundance eliminated)	−0·655
	Abund.—Size (temperature eliminated)	+0·221
Bed no. 2:	Temp.—Size (abundance eliminated)	−0·451
	Abund.—Size (temperature eliminated)	−0·016

These results, of which the −0·655 and −0·451 values are significant, indicate that the temperature influence on size is dominant over that of crowding in this series of observations.

Most of the *P. severini* emerged during the treatment months and, therefore, size may have been influenced by some factor other than temperature or population pressure. The temperature effect upon the flies in the bed cannot be studied, as a mixed lot of flies from the

two beds were measured, the numbers being generally insufficient to allow of separate measurement. It is possible that in this case interspecific competition is an important factor as the numbers of *P. severini* at Huddersfield during the period of survey have been small in comparison with those of *P. alternata* and at Knostrop it is an abundant insect only in the late spring and then remains scarce at trapping site no. 3 where Lloyd *et al.* (1940) have shown that interspecific competition is most acute. As the Huddersfield flies were not measured from separate beds it is not possible to examine the influence of *P. alternata* density on *P. severini* size by the method of partial correlation and the complicated data from Knostrop are as yet insufficient.

Examination of Table 9 which gives the wing length of *P. alternata* at Huddersfield on beds nos. 1 and 2 and the average size for the three positions at Knostrop may help in an understanding of crowding. This suggests at certain points that intraspecific crowding causes smaller flies. Contrasting beds nos. 1 and 2 it will be noticed that up till June the flies from the more sparsely populated no. 2 are larger than those from no. 1. After this however, probably owing to the reduction of population pressure on no. 1 by treatment, the flies increase in size and throughout the summer there is very little difference in size between the inhabitants of the two beds. However in November and December, when no. 1 is again exceeding no. 2 in output, the same size ratio is present, i.e. the flies on no. 2 are larger. Even though the temperatures of the two beds are slightly different, no. 2 being colder by 1–2° C., this difference is not likely to cause the discrepancies in size, which are generally more than 4%.

TABLE 9. *Bed temperature record, Huddersfield and Knostrop. Monthly averages,* ° C. Psychoda alternata, *Huddersfield and Knostrop. Female monthly wing measurements in mm.*

	Huddersfield, no. 1		Huddersfield, no. 2		Knostrop	
Month	Temp. ° C.	Wing length	Temp. ° C.	Wing length	Temp. ° C.	Wing length
Mar. 1938	12·0	2·37 ± 0·014	10·2	2·42 ± 0·007	10·9	2·36 ± 0·004
Apr.	12·6	2·13 ± 0·028	11·2	2·27 ± 0·012	10·6	2·18 ± 0·014
May	14·9	2·10 ± 0·014	13·7	2·18 ± 0·029	12·0	2·16 ± 0·009
June	15·8	2·26 ± 0·019	15·0	2·27 ± 0·014	14·6	2·11 ± 0·019
July	17·5	2·16 ± 0·015	16·8	2·17 ± 0·008	15·4	2·13 ± 0·015
Aug.	17·5	2·06 ± 0·011	17·4	2·09 ± 0·009	16·8	2·02 ± 0·014
Sept.	16·8	2·06 ± 0·009	16·5	2·04 ± 0·013	15·9	2·23 ± 0·013
Oct.	12·7	2·23 ± 0·008	12·3	2·17 ± 0·013	12·8	2·19 ± 0·012
Nov.	12·2	2·12 ± 0·015	10·1	2·23 ± 0·014	11·8	2·44 ± 0·012
Dec.	9·1	2·13 ± 0·015	7·8	2·28 ± 0·012	7·8	2·38 ± 0·035
Jan. 1939	7·6	2·47 ± 0·017	6·9	2·43 ± 0·026	6·3	2·47 ± 0·047
Feb.	9·7	2·38 ± 0·013	8·7	2·41 ± 0·023	9·3	2·26 ± 0·041
Mar.	10·3	2·45 ± 0·012	9·5	2·49 ± 0·014	8·4	2·59 ± 0·080

In the period March–August 1938 *P. alternata* and *P. severini* counted together were trapped in the following ratio on bed no. 1 and bed no. 2 of Huddersfield and on the Knostrop bed—369 : 428 : 100—and the wing lengths of *P. alternata*, monthly averages, were in the ratio 101·12 ± 1·465 : 103·40 ± 0·304 : 100. That is, the flies at Huddersfield on bed no. 2 were significantly larger than at Knostrop and those on bed no. 1 were also larger on the mean but not so uniformly larger as on bed no. 2. In the period September 1938–February 1939 the corresponding ratios for numbers were 2466 : 1609 : 100 and for wing length, monthly means, 93·57 ± 2·444 : 94·80 ± 1·421 : 100. That is, on both Huddersfield

beds the flies were significantly smaller than at Knostrop on the monthly means. In the more varied conditions of the competition at Knostrop in the spring and summer *Psychoda* spp. are relatively more abundant than they are in the autumn and winter when they become much scantier relative to the increasing dominance of the chironomids (Lloyd *et al.* 1940). It may be supposed that the intraspecific competition amongst *Psychoda* will be keener in the March–August period because they are then much more abundant than from September–February when their numbers are reduced in various ways by the chironomids, *P. severini* at least being almost totally eclipsed. (Reliance in this argument has been laid on the dimensions of *P. alternata* because *P. severini* becomes so scarce seasonally at Knostrop that a continuous satisfactory estimate of its size cannot be obtained from the trapped flies for six months of the year.)

These facts certainly suggest that the broad interspecific competition at Knostrop affects in particular numbers, but the more intimate and restricted competition at Huddersfield amongst an exclusive population of *Psychoda* shows its influence more on the size of the individuals though of course numbers must also be affected.

It is interesting to note that the effects of size are such that they tend to balance and stabilize the population density. If the temperature and amount of available food were favourable and constant, this balanced condition might come into being. It has been proved that the number of eggs laid and the size of the parent are directly proportional and hence a certain number of small flies produce as many eggs as a smaller number of large flies. Therefore, under ideal conditions of temperature and food, we should eventually reach a population of constant density and size. This is prevented in the beds by the varying conditions which influence survival rates, but the knowledge is of interest in considering possibilities of fluctuations in abundance and hence competition.

Summary

The insect fauna of the Huddersfield Corporation sewage bacteria beds has been studied and compared with that of Knostrop (Leeds). The structure and working of the beds together with the character and treatment of the sewage are described.

The dominant element of the Huddersfield fauna is *Psychoda alternata* and its abundance is attributed to the absence of chironomids which compete with it at Knostrop. Its larvae inhabit particularly the topmost zone of the bed which at Knostrop is appropriated by *Metriocnemus* species which therefore takes from the *Psychoda* not only the best zone but also reduces the total habitable depth. Its abundance at Huddersfield is such that treatment with creosote is undertaken to reduce its numbers and this is shown to be more effective on fine surfaced beds which probably retain the fumes for a longer period.

By laboratory culture, temperature and crowding are shown to affect the size of *P. alternata* and *P. severini*, wing length being employed as a criterion. Flies from the beds have been measured systematically and the size of both species of *Psychoda* has been found to fall steadily from winter to midsummer and then rise again. Temperature and crowding are both potent factors in this but the former is dominant. An artificial grouping of the data indicates that a rise of 2° C. in mean temperature entails approximately a reduction of 4% in wing length.

It was found that the size of the female parent does not influence the size of the offspring but small flies lay fewer and rather smaller eggs than large flies. It is suggested that this fact might stabilize the population at a given temperature and with unlimited food. There are strong indications that interspecific competition tends to reduce numbers but intraspecific competition has a more pronounced effect upon size of the individual.

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OBSERVATIONS ON A DISEASE OF *SCILLA CAMPANULATA* AIT. DUE TO THE STEM AND BULB EELWORM, *ANGUILLULINA DIPSACI* KÜHN

By E. E. EDWARDS, M.Sc.

Advisory and Research Zoologist, University College, Cardiff

(With Plates 17 and 18)

Introduction

In a recent paper (Edwards, 1937*a*) on a disease of primulas caused by the stem and bulb eelworm, *Anguillulina dipsaci* Kühn, it was indicated that the first serious outbreak was discovered in the early part of 1937 on specimens of *Primula japonica* A. Gray, which formed part of a large collection of *Primula* spp. growing at the Educational Gardens, Singleton Park, Swansea. Later, the disease became so virulent that by the end of that summer all the plants, irrespective of their species, had succumbed to the attack. Further attempts at the cultivation of primulas in this border had, therefore, to be discontinued, and in the autumn of 1937 the ground was well dug and planted with bulbs of *Scilla campanulata* Ait. At first the scillas made normal growth, but early in April 1938 numerous individuals were exhibiting marked symptoms of nematode attack and, upon microscopic examination showed, in their aerial parts, heavy infections of *Anguillulina dipsaci* in all stages of development. Subsequent inspections revealed that most of the other scillas growing in the border were also attacked.

While conclusive experimental data are not yet available regarding the origin of the infestation of *Scilla campanulata* by the stem and bulb eelworm in the present instance, circumstantial evidence points to the conclusion that a natural transfer of the strain parasitic on primulas had occurred. The border in which the bulbs of *S. campanulata* were planted had never previously grown any species of *Scilla*. It had been part of a field formerly under permanent pasture up to 1935 when it was brought into cultivation and planted a few months later with various species and varieties of *Primula*. The *Scilla* bulbs planted in 1937 in this infected border formed part of a stock which had been successfully grown for several years some distance away in another section at this centre. The remainder of the bulbs still growing in the original position in 1938 were found entirely free of any infestation by the eelworm. The inference that the scillas became infested subsequently to planting in the border was supported by the observations made on the development of the infection within the plants. In every case investigated, the disease manifested itself in the first instance in the aerial parts of the plant. The infection of the bulbs themselves took place much later in the year by the downward migration of the eelworms from the neck region and, despite extensive and detailed inspections, the parasite was not found deeply situated in any bulb until towards the end of the growing season.

As far as can be ascertained, no previous authentic record of the natural occurrence of *Anguillulina dipsaci* on any species of *Scilla* exists, although Ritzema Bos (1892) had succeeded in setting up infection experimentally in healthy *S. sibirica*, *S. campanulata* and *S. cernua* by using the worms from hyacinth and from rye. The symptoms of infection

produced in the scillas were said to be very similar to those shown by the hyacinth (*Hyacinthus orientalis*) when affected with the "Ring Disease" due to *Anguillulina dipsaci*. Ritzema Bos also indicated that prominent growers of bulbs in Holland were of the opinion that a "Ring Disease" actually occurred at times in scillas on their own plantations. Apart from this statement, no evidence was produced to substantiate such a belief or to demonstrate the precise cause of the disease. No other occurrence of *A. dipsaci* on scillas seems to have been recorded except by Ramsbottom (1918) who, in the course of an account of his investigations on the disease caused by this parasite in narcissi, briefly reported that "*Scilla nutans* has also been successfully cross-inoculated with the narcissus strain of *Tylenchus* (*Anguillulina*), and a number of other crops are under observation".

Symptoms of attack

The effects of infection by the stem and bulb eelworm in *Scilla campanulata* vary greatly with the intensity of attack but throughout its progress the symptoms are characteristic. The diseased plants never make good growth so that they can be described as unthrifty in appearance even if they succeed in attaining maturity and in flowering. Adults, larvae and eggs of the parasite are usually found in abundance, both in the aerial parts and in the bulb of affected plants.

Foliage symptoms. The leaves are lighter green, ranging in some cases almost to silvery grey as opposed to the deep green of normal plants. Occasionally, they exhibit the characteristic twisting and distortion so typical of infestation by *Anguillulina dipsaci* in narcissus and many other plants (Pl. 17, fig. 2). Extensive decaying of the tissues at or just below ground level is often evident, particularly about the time when the foliage is withering from natural causes. Affected leaves are usually thickened, soft and spongy in texture, and instead of standing moderately erect around the flower stem, are limp and flaccid, and in extreme instances lie prostrate on the ground even in young plants (Pl. 17, figs. 1, 5).

The most common symptom of diseased leaves is the occurrence on them of light-coloured pustules or blister-like swellings (Pl. 17, figs. 1, 2). These pustules in *Scilla campanulata* are reminiscent of the "spickles" commonly seen in narcissi attacked by *Anguillulina dipsaci* except that they are somewhat less swollen and, in consequence, less readily felt when the affected leaves are drawn between the finger and thumb. The lesions begin as pale green spots which soon develop into grey, and ultimately, white areas, usually surrounded with a yellowish peripheral zone which gradually merges into the green of the healthy tissue. They increase very rapidly in size and the epidermis over them often splits and turns brown. Frequently, the infection first develops at some point in the midrib and the immediately adjacent tissues, later spreading in all directions, and in severe attacks the pustules may occur in large numbers over the whole leaf, giving it a distinct mosaic appearance.

Flower symptoms. The flower stems are usually much dwarfed, the degree of retardation depending on the intensity of attack (Pl. 17, fig. 3). Even when infested plants succeed in flowering, the stems are spindly and brittle, or swollen and soft. In either case, marked chlorosis, typical twisting and distortion of the stems are also frequent symptoms (Pl. 17, fig. 3). The flower stalk of a seriously distorted plant is often curved through two complete right angles. In advanced stages of attack, the basal regions of the flower stems are, like those of the leaves, particularly prone to injury. Frequently, the plants grow vigorously at first, and then succumb rapidly to the attack, the aerial parts suddenly rotting off at the base, although they may have previously exhibited no definite sign of infestation. The decaying may also occur much higher up the stalk, often at the base of the inflorescence.

As with the foliage, the characteristic pustules are generally present on the flower stems of heavily infested plants (Pl. 17, fig. 2*p*). Such stems often split longitudinally, or sometimes transversely (Pl. 17, fig. 2*s*). As a rule, the numbers of individual flowers are also reduced, in extreme cases to about three poorly developed specimens (Pl. 17, fig. 2). The inflorescence may be unusually open or loose, or, more often, abnormally close and compact (Pl. 17, fig. 2). An inflorescence may show well-developed, as well as deformed flowers, or all abnormal ones. Deformed flowers, besides being undersized, may be found with much thickened and dwarfed pedicels and perianth diminished to a mere vestige. In severe infections the different components of the gynoecium may also show extreme forms

of malformation. Frequently, a badly attacked plant, however, produces an inflorescence of normally shaped flowers except that they are few in number, somewhat small and pale in colour compared with those of healthy plants. On such plants the flowers generally die prematurely; if any seed is set, a large proportion of it is sterile.

Bulb symptoms. In the early stages, small, pale yellow lesions are seen, situated mainly on the apical region of the bulb. The lesions are at first slightly raised, but later they become sunken and the margin more distinct. In advanced conditions the affected parts are irregular in outline, definitely sunken, and deep brown or reddish brown in colour (Pl. 18, fig. 3). Direct entry by the parasite into the bulb itself is, however, not always restricted to the apical region, and sometimes the sides or lower halves of bulbs may be seriously invaded while the upper parts remain free (Pl. 18, figs. 1, 2). As the attack advances, the lesions tend to spread over the greater part of the bulb which, when cut across, often shows that the infection remains superficial for some considerable time, frequently until the following season (Pl. 18, figs. 4, 5). The singular slowness with which the attack progresses in the surface tissues of the bulbs is due to the inability of the worms to penetrate freely the walls of the outermost scales.

Frequently, bulbs that appear to be healthy on the outside may disclose internally well-marked symptoms of infection. It is then generally found that the parasites gained access into the interior by wandering downwards from the aerial parts to the corresponding tissues within the bulb. Once an entry in this manner is effected into the bulb, the eelworms normally make, at least subsequent to the period of active development by the leaves and flower stem, a very rapid downward migration to the basal plate. The tissues at the base of the scales may then be reduced to a putrid, discoloured mass, discernible on the outside of the bulb as a sunken, deep-brown or reddish brown necrotic area, often completely encircling the basal plate (Pl. 18, fig. 6). On cutting through such an infected bulb, the damaged areas are seen as discoloured streaks gradually disintegrating into brown or blackish decayed matter (Pl. 18, fig. 7). In longitudinal section, the discoloured streaks can usually be traced in the earlier phases of attack as a narrow band following the course of the flower stem to the base of the bulb (Pl. 18, fig. 6), but sometimes in the form of narrow strands traversing the scale-leaves lengthwise. As the infection proceeds, more and more of the scales become invaded until, finally, a general putrefaction, aided by secondary invaders, both animal and vegetable, sets in and spreads rapidly throughout the bulbs (Pl. 17, fig. 4).

Very occasionally, infested bulbs in an intermediate stage of decay exhibit, when cut transversely, one or more incomplete rings of brown, disorganized tissue, lying between still white and healthy ones, a condition somewhat resembling that produced by *Anguillulina dipsaci* in narcissus bulbs. These discoloured rings seldom however occur in such well-defined, concentric bands as in the narcissus, the eelworms being apparently capable of penetrating freely through the inner scales from one to another in a radial direction. Frequently, rotting of the innermost scales of bulbs proceeds simultaneously with the spread of external symptoms and "arms" of diseased tissue containing the parasite can then be traced from the dead core through the flesh to the lesions on the surface scales (Pl. 18, fig. 8).

Affected scales, besides turning dark in colour, usually become thickened owing to hypertrophy of the cells. These changes are also frequently accompanied, unless a rapid decay intervenes, by an increase in size of the bulbs compared with that of healthy ones, and also by cracking, usually lengthwise, of the outer fleshy scales (Pl. 18, figs. 10, 12, 13). As a rule, the splitting extends deeply into the interior of the bulbs and in such cases the young shoots, instead of developing normally and appearing at the apex of the bulb, often start growth abnormally early and emerge through the cracks at an unusual position, most frequently at the side of the bulb but sometimes at a point near the basal plate or in the neck region (Pl. 18, fig. 12). Not infrequently, affected bulbs when left in the ground, also show the curious behaviour illustrated in Pl. 18, fig. 11, the sprout having at first grown downwards and emerged through the basal plate. It is generally found in such cases that the basal plate has either broken away from the remainder of the bulb or, more commonly, is in an advanced state of decay, as well as the lower part of the scales.

Between the commencement of an attack and the death of the bulb, at least six months usually intervenes, even when an infection takes place in April or May. In light infestations, death may not occur for one or more years, especially if the attack at first is confined to the outermost scales. A distinctive feature of infestation is also the depressing effect on the rate of multiplication of the bulbs. Although as a general rule lightly affected bulbs manage to thrive for a time and become larger in size than healthy ones, they seldom show any tendency to form offsets. The actual loss that may follow an attack is shown by the following figures. Out of 500 healthy bulbs of *Scilla campanulata* planted in infected ground in September 1937, and allowed to grow undisturbed in the soil for two seasons, only 167 bulbs were lifted in October 1939, the remainder having succumbed to attack by *Anguillulina*

dipsaci. All these bulbs were affected by the parasite in varying degrees and only 14 new bulbs or offsets had developed in 1939. On the other hand, a collection of 200 healthy bulbs from the same stock grown at the same centre under similar conditions, but in non-infested soil, remained free from infection and, on lifting in October 1939, two seasons after planting, showed an increase of approximately two and a half times in the number of bulbs, the actual figure being 514.

A significant feature associated with the infestation at this centre was also the marked increase in size of many of the bulbs compared with the healthy ones. In an attempt to assess this difference, three separate lots, each consisting of 30 bulbs, were picked from both the infested and healthy samples of bulbs. The bulbs were selected at random but avoiding the very small and badly decayed specimens. They were then air-dried and weighed (Table 1).

TABLE 1. *Weight of different samples of healthy and infested bulbs of* Scilla campanulata

Each sample—30 bulbs

	Healthy oz.	Infected oz.
Sample A	29·50	69·50
Sample B	34·25	62·50
Sample C	27·75	53·25
Av. weight per sample	30·75	61·75
Av. weight per bulb	1·02	2·06

While the healthy bulbs when fully developed were remarkably uniform in size and weight, each approximately 4 cm. in diameter and 30 g. in weight, the infested ones showed extremely wide variations in these respects, ranging up to about 7·5 cm. across and 108 g. in weight (Pl. 18, figs. 9, 10). A striking peculiarity of the abnormal diseased bulbs was also the severe cracking and deformed appearance which was most evident in the larger ones (Pl. 18, figs. 12, 13). The least affected bulbs were but slightly misshapen and the fissures formed in the surface layers were comparatively small and narrow (Pl. 18, fig. 10). In typical cases, however, the bulbs were badly misshapen, exhibiting wide fissures or cracks, irregular in outline, exposing brown necrotic tissues extending deeply into the interior (Pl. 18, fig. 12). In addition, the lower extremity of the scales and the basal plate of some of these bulbs were dead or moribund and the root system absent or very poor, whilst in many of the others the outermost scales covering the apical region of the bulb were largely disintegrated (Pl. 18, fig. 13). In more advanced instances, the decaying and splitting had continued still further in the tissues, causing the entire bulb to fall apart with only the small, developing buds remaining (Pl. 18, fig. 14).

BIOLOGY OF THE PARASITE

1. *Entry into the host*

All parts of *Scilla campanulata*, both above and below ground, are subject to attack from *Anguillulina dipsaci*, with the exception of the root system. Entry by the eelworm into the plants can be effected at several points, namely:

(1) In the apical region of the shoot as it pushes through the soil.

(2) At the base of the flower stem and leaves.

(3) At the neck of the bulb itself where the shoot emerges, particularly where splitting of some of the outer scales has occurred.

(4) At the side of the bulb, most frequently half-way up, sometimes higher, sometimes lower, but never through the basal plate. This mode of entry was only noted on rare occasions.

2. *Movement within the host*

Once the leaves and flowering structures are entered the eelworms continue to feed and multiply with great rapidity from early April until the end of June, plants by then having reached full maturity. Plants infested earlier than the beginning of April are rarely found to

show, under outdoor conditions, any appreciable increase in the numbers of eelworms present. Eelworms entering plants in active growth are carried far above their site of entry into the rapidly growing aerial portions. As the eelworms increase in numbers, chlorosis, thickening, distortion and finally death of the affected parts occur, the parasites migrating into fresh tissues as the decay spreads. Ultimately, large numbers of the nematode leave the plant and enter the soil unless this is prevented by dead or dried tissues, in which case, if trapped in moist decaying portions, they die in a very short time. If, on the other hand, the infested parts dry up, the eelworms become quiescent in which state they can remain viable for a considerable period, becoming active again on getting into contact with the soil or any moist environment. For example, infested foliage in a dried, shrivelled-up condition, was brought into the laboratory in May 1938 and stored in a stoppered glass phial. In January 1940, small portions of the material were placed in water in glass capsules and some hours later it was found that many of the eelworms present had, after a period of 19 months, revived and resumed active movement. It is probable that the parasite can persist and retain its vitality in dry foliage of *Scilla campanulata* for several years, judging from observations on the narcissus strain (Hodson, 1932).

As a result of observations in 1938, it was found that no appreciable infestation of the bulb itself takes place in infected soil until some weeks after the blossoming period and when the foliage has begun to die. At this stage, contrary to our knowledge of *Anguillulina dipsaci* in narcissus, there is an apparent downward migration into the bulb of large numbers of the nematode from the aerial parts especially from the flower stem (Pl. 18, fig. 6). Experimental confirmation of this apparent downward movement of the eelworms was obtained by cutting away the aerial parts of infected plants close to the neck of the bulb, at different intervals between the full blossoming period and the dying down of the foliage and flower stem. In general, the degree of infestation of the bulbs towards the end of August was in direct relation to the date at which the aerial parts were removed, being most severe where it was delayed until the foliage had become completely withered.

The rate at which an attack progresses within bulbs depends partly upon the numbers of eelworms that originally enter the bulb, and partly upon the degree of infestation of the flower stem and its inflorescence. The most rapid destruction of the bulbs almost invariably follows heavy attacks of the flowering structures. The spread of the parasite downwards within the bulb along the old floral stalk into the tissues adjoining the base plate is then exceedingly rapid. Here the eelworms become particularly active, the succulent tissues at the base of the scales where these border the basal plate being apparently highly favourable for growth and reproduction. In contrast to conditions in the narcissus, the membranous walls separating the inner scales in the bulb of *Scilla campanulata* offer little or no resistance to the passage of eelworms from one scale to another (Pl. 18, fig. 8). Generally, the rate of progress of the eelworms which have penetrated directly into the bulbs is, on the other hand, relatively slow. The parasites often persist for several months in the surface tissues and invade a wide area without penetrating deeply into the bulbs. The period during which an infestation is confined to the outer tissues seems to be longer when the infected area is at the side than when it is at the neck of the bulb (Pl. 18, figs. 1, 3). The inference is that the eelworms are unable to penetrate freely through the walls of the external scales and, in consequence, they tend to work around the bulb, leaving the tissues dead behind them (Pl. 18, fig. 5).

3. *Persistence in the seed*

The occurrence of *Anguillulina dipsaci* in the flower and ovules of infested plants, the manner of infestation and the length of time during which the parasites in dry seeds can retain their vitality are matters of considerable practical and theoretical interest. Since very little information has, hitherto, been available, advantage was taken of the attack of *A. dipsaci* on *S. campanulata* in South Wales to study these problems. It has already been found that where infection has reached the basal region of the bulb, the embryonic foliage and flower tissues laid down for the following season have generally been invaded by the nematode (Pl. 18, fig. 6). Great numbers of all stages of the parasite are often present, especially in the tissues of the inflorescence. It would seem that invasions prior to, or at any early phase in, the differentiation of the flower bud invariably result in complete atrophy of the floral elements, whilst later attacks lead to inflorescences of various degrees of deformity. Thus flower shoots infested after the commencement of the actively growing period, by invasion either from the ensheathing bulb-scales (Pl. 18, fig. 4) or later from the infected soil, frequently retain their normal appearance except for lack of vigour and reduction in number and size of individual blooms.

That ready infestation of the aerial parts takes place as these are pushing upwards through infected ground was amply demonstrated by transplanting in February 1939 a number of healthy bulbs of *Scilla campanulata* into infected soil. These bulbs were free of eelworm when they were planted in steam-sterilized soil in the preceding autumn. They were set at a depth of 4 in. in the infected ground and, at the time of transplanting, the sprout was in most instances approximately 2 in. in length. Seven weeks later when the shoots were a few inches above soil level *Anguillulina dipsaci* was almost invariably found to be present in the leaves and especially in the tissues of the inflorescence. No such infection was found in control plants grown under identical conditions in non-infected ground. The nematodes present in the attacked scillas were comparatively few in number, and in the pre-adult infective stage except for a very small proportion which had reached sexual maturity, presumably since entering the plants. No free eggs were seen in the tissues of any plants until the first week in April.

At intervals in 1939, inflorescences of typically affected plants were teased out under a binocular microscope, and the individual components examined for the presence of the parasite. It was found that the nematode frequently occurs in large numbers in all stages, from egg to adult, in the main flower stem, pedicels, petals, stamens and different parts of the gynoecium. Ovules of diseased plants, at different stages of maturity, were also dissected and an estimate made of the numbers of the parasite present in each. Newly formed seeds from such plants were often found to contain many living adults of both sexes, as well as numerous eggs and larvae. For instance, the ripening seeds or ovules removed from the seed-capsules of heavily infested plants some 3–5 weeks after the blossoming period in 1939, yielded 2–19 adults, 6–35 larvae and 29–242 eggs per ovule.

Conclusive evidence has also been obtained that *Anguillulina dipsaci* can remain viable both upon and within the seed of *Scilla campanulata* for at least 18 months. Seed capsules which had been gathered in July 1938 from moderately infected plants and subsequently kept in the laboratory in a dry state were, in January 1940, pulled apart and the seed put into water. Within a few hours quiescent larvae present upon the seed-coat had uncoiled and

resumed active movement. Full grown adults were also present but these remained quite motionless even after several days and finally disintegrated. After 24 hr. soaking, a number of the seeds were washed under running tap-water at 120° F. to destroy any larvae that might be adhering to them, and then placed in water in glass capsules. Afterwards the seeds in some of the capsules were cut open and, on teasing out their contents, pre-adult larval forms in a coiled-up condition were, in 38% of the cases, liberated into the water. The number of larvae ranged from 2–19 in each ovule. Quite 70% of the worms became actively motile within 24 hr. of liberating, and had thus remained viable for a period of 18 months. Despite the presence of the worms, a high proportion of the infested seed appeared undamaged. In some cases a few sexually matured parasites were also set free on dissection but none showed signs of motility, even on warming. None of the seeds, however, contained eggs and, in no instance, were any worms found in the capsules, left as controls, with the washed, non-dissected seeds.

4. *Powers of adaptation to other host plants*

In order to determine whether the race of *Anguillulina dipsaci* parasitic on *Scilla campanulata* in South Wales is capable of attacking other plant species, an attempt was made to transfer the nematode to other hosts under controlled conditions. Earthenware flower pots of 11 in. diameter were filled with soil and sunk into the ground to within 2 in. of the rim in an open-air, wire-covered enclosure. The soil used, the same in all pots, except the controls, was taken from a section of a flower border at the Educational Gardens, Singleton Park, Swansea, where *Scilla campanulata* had suffered severely from the depredations of *Anguillulina dipsaci* in the preceding season. The controls were filled with virgin soil assumed to be free from *A. dipsaci*. As all the plants grown in the controls remained free from attack with the exception of *Phlox paniculata*, it may be inferred that this assumption was justified. There is reason to believe that the phlox was already infected by the parasite when planted. Fifteen potential hosts of *Anguillulina dipsaci* were then planted, eight pots, including two controls, being used for each plant species. The plants tested were:

(1) Cultivated narcissus (*Narcissus incomparabilis*).
(2) Cultivated tulip (*Tulipa* sp.).
(3) Hyacinth (*Hyacinthus orientalis*).
(4) Cultivated scilla (*Scilla campanulata*).
(5) Wild bluebell (*S. nutans*).
(6) Bulbous iris (*Iris xiphioides*).
(7) Cultivated primula (*Primula japonica*).
(8) Herbaceous phlox (*Phlox paniculata*).
(9) Potato (*Solanum tuberosum*).
(10) Onion (*Allium cepa*).
(11) Rye (*Secale cereale*).
(12) Oat (*Avena sativa*).
(13) Culinary pea (*Pisum sativa*).
(14) Broad bean (*Faba vulgaris*).
(15) Red clover (*Trifolium pratense*).

The bulbs chosen for series 1–6 were from stocks known to have shown no signs of attack by *Anguillulina dipsaci* over a number of years. In each pot 8–12 bulbs were planted,

depending on the species. The seedling primulas used in series 7 formed part of a batch of seedlings raised from seed which had been sterilized for the control of *A. dipsaci*, according to a method recently evolved by the writer for this purpose. Experience gained in previous work indicated that such a precaution was necessary in obtaining young primulas for infection experiments since the biological race of this nematode attacking primulas is readily carried by their seed (Edwards, 1937*a*). The young phlox plants for series 8 were from a commercial source and claimed to be "absolutely free from infestation by the Phlox Eelworm (*A. dipsaci*)". The potato tubers planted in series 9 were Scotch in origin and showed no signs of attack by *A. dipsaci* or any other pest. All the other plants in series 10–15 were grown from seed obtained through the ordinary trade channels and sown directly into the pots.

Whenever required, watering was done with tap-water to minimize the chances of accidental infection and in such a way as to approach as closely as possible field conditions. The plants in all series, including the controls, were periodically examined from the time of their appearance through the soil until full maturity, first, with the naked eye for any indications of attack by *A. dipsaci* and then microscopically for the presence of nematodes whenever there was any evidence to suspect them. In addition, a few plants, irrespective of the presence or absence of signs of attack, were removed at intervals from both the affected and non-infested soils in all series for investigation in the laboratory. In each series, some plants were carefully teased out under a binocular microscope and examined for the parasite, whilst others or portions of them, depending on the size and stage of growth, were submitted to Goodey's (1937) method for staining eelworms within plant tissues by means of Scarlet R.

All the host plants grown in the control pots remained healthy in appearance throughout the season and no eelworms were seen within their tissues except in *Phlox paniculata*. Despite the claim of freedom from eelworm infection, which was made by the nurseryman who supplied the young phlox plants, all developed to some extent symptoms of attack by *Anguillulina dipsaci*. Large numbers of the parasites were also found in their aerial parts and no difference could be detected at any time between the plants in the two different soils.

Several of the host plants when grown in pots containing infested soil, on the other hand, showed invasion of their tissues by *A. dipsaci*. As expected, infection of *Scilla campanulata* occurred readily with disastrous results, great numbers of the nematode in all stages of development being found in the foliage and flower structures. Little or no evidence of entry into the bulbs themselves was observed until after the full blossoming period. *S. nutans* became infected at an early date but the rate of reproduction of the eelworm throughout the year was definitely slower and the injury to the plants much less than in *S. campanulata*. Although the nematode was found in small numbers in the leaves of a few narcissi during their early growth, no deleterious effects were noticed on these plants, and all attempts, after the first ten weeks, to find any worms within their tissues failed. All the eelworms found were exceedingly sluggish and from their general condition it seemed that they had not fed since entering the plants. In the case of the bulbous iris, a few nematodes were found in three instances, at the time of lifting, near the edges of small cracks present at the apex of the outermost fleshy scales of the bulb. No reproduction took place and the infection eventually died out. Early examinations showed eelworms to be present in the young leaves of hyacinths, but no apparent damage was done. Later, nematodes were also noted on two occasions in the tissues at the base of the scape just above ground level, as well as in partially

decayed areas in the neck region of the bulb itself. This decay was by no means typical of the damage usually caused by *Anguillulina dipsaci* in bulbs, and, as with infestation of the foliage, the parasite failed to become established. In contrast, not a single eelworm was encountered in the tulips examined. Invasions of *Primula japonica* freely occurred, the basal portions of the leaves and flower stems being the positions primarily entered. In general, the attack was typical of that normally associated with an infection by *Anguillulina dipsaci* on this plant (Edwards, 1937*a*). In one potato plant a number of living and dead nematodes was discovered in a slightly discoloured area in the axil of a leaf, soon after the sprout had come through the soil. No eggs were seen and no further invasions could be detected until early August. Comparatively large numbers were then located in the tissues of the stem a little below ground level, the plant itself having prematurely died due to decay at this point. Again no eggs were detected. In the onions and broad beans, isolated individuals were occasionally found but only at the seedling stage. In the remaining host plants, no evidence of the parasite was recorded at any time within their tissues.

Earlier in this paper, circumstantial evidence was produced indicating that a natural transfer of *A. dipsaci* from *Primula* spp. to *Scilla campanulata* occurred in South Wales in 1937–8. The results of the cross-infection experiments provide further evidence since a typical attack by *Anguillulina dipsaci* was obtained in healthy seedlings of *Primula japonica* planted in soil which had grown diseased *Scilla campanulata*. It should, however, be noted that primulas had been grown in the flower border where the soil for these experiments was obtained, up to June 1937, when this practice had to be discontinued, due to the ravages of *Anguillulina dipsaci*. Some three months later healthy bulbs of *Scilla campanulata* were planted in the border, but early in 1938 most of the plants exhibited a severe attack by *Anguillulina dipsaci*. Soil closely adjacent to badly infected bulbs was taken in August 1938 for the host range experiments, and the healthy primula seedlings were actually planted in the pots containing this soil on the 20th October 1938, some 16 months after the border had been cleared of infested primulas. In view of such a short intervening period it is conceivable that the infection of the primula seedlings in these experiments was connected with the occurrence of natural infection on *Primula* spp., rather than that on *Scilla campanulata*. To assume such association would imply that two strains of *Anguillulina dipsaci* existed together in the soil, one capable of attacking primulas and the other of infecting scillas, and that the former strain can live in moist soil for at least 16 months in the absence of its host plant. From the findings of van Slogteren (1920) and Goodey (1931) on the narcissus race and Edwards (1936) on the potato strain, there seem to be definite reasons for rejecting this hypothesis since it entails the persistence of the parasite as a living infective organism in moist soil for a period exceeding well over 12 months. The possibility of some weed having formed a reservoir of infestation in the meantime can be ruled out as all weeds found growing in the flower bed from which the soil for the experiments was taken have been under close observation since the early part of 1937 when the first outbreak on primulas made its appearance. Despite very careful search throughout both 1937 and 1938, no instances of attack upon any weed were recorded.

From the evidence at present available it would seem that the parasites from *Scilla campanulata* and *Primula* spp. are reciprocally infective and constitute a rather highly specialized strain. When given access to plants liable in South Wales (Edwards, 1937*b*) to serious injury from *Anguillulina dipsaci*, including the narcissus, bulbous iris, hyacinth,

onion, potato, oat, broad bean and red clover, the parasites failed to become established and reproduce. It must not be inferred, however, at the present stage of our knowledge that, where infestations of *Scilla* spp. or *Primula* spp. occur, any plant species which gave negative results in the susceptibility trials already described, can be grown without risk of attack. It is significant that entry into plants other than scillas and primulas was frequently effected, and, in such cases, as in the narcissus and hyacinth where the worms persisted within the tissues for periods extending to months, it is conceivable that adaptation to the new hosts might eventually be accomplished.

Summary

1. Attention is drawn to the occurrence in Britain of a serious disease of *Scilla campanulata* Ait. due to the stem and bulb eelworm, *Anguillulina dipsaci* Kühn. This is the first record of the disease and evidence is submitted to show that the infection probably originated by planting scilla bulbs in soil heavily infested with the biological race of *A. dipsaci* parasitic upon plants of the genus *Primula*.

2. The characteristic symptoms of the disease are described in the foliage, flower structures and bulbs of attacked plants when grown under natural conditions.

3. The biology of the parasite is discussed with special reference to its mode of entry into the host, movement within the tissues of the plant and persistence in viable condition both upon and within the seed of infected plants.

4. The results of infection experiments with other plants are also presented in detail.

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EXPLANATION OF PLATES 17 AND 18

Plate 17

Figs. 1, 5. Young plants of *Scilla campanulata*. In fig. 1 the plant exhibits a characteristically limp and flaccid appearance due to a heavy infestation of its tissues by *Anguillulina dipsaci*. Fig. 5 is that of a healthy plant (see p. 423).

Fig. 2. *Scilla campanulata* seriously attacked by *Anguillulina dipsaci*. Note the stunted growth of the entire plant, spickles or pustules on the foliage (*p*), deformed and twisted leaves, distorted flower stem with its epidermis split at right angles to the longitudinal axis (*s*), and a much reduced inflorescence consisting of shrivelled flower buds (see pp. 423–4).

Fig. 3. Typical inflorescences of *Scilla campanulata* when attacked by *Anguillulina dipsaci*. Note the presence of spickles or pustules (*p*), distorted and twisted flower stems (*b*, *e*), uneven distribution of the bloom (*a*, *b*, *c*), marked reduction in size and numbers of individual flowers (*b*, *d*, *e*) and in extreme case (*c*) the entire inflorescence having wilted and died prematurely (see pp. 423–4).

Fig. 4. Bulb of *Scilla campanulata* cut longitudinally to show almost complete disintegration of all its tissues due to *Anguillulina dipsaci* (see p. 424).

PLATE 18

Figs. 1–3. Bulbs of *Scilla campanulata* showing sharply localized lesions following the direct entry by *Anguillulina dipsaci* into the outermost scales at side (fig. 1), base (fig. 2), and apex (fig. 3) of the bulb (see p. 424).

Figs. 4, 5. Bulbs, essentially similar to those in figs. 1–3, cut longitudinally to show that the parasites are unable to penetrate freely the walls of the external scales, and, in consequence, tend to confine their activity to the outer tissues and invade a wide area on the surface of the bulb (see pp. 424, 426). Fig. 4 illustrates a form of infection that invariably leads to invasion of the developing aerial parts as these are making their way through this region (see p. 427).

Fig. 6. Attacked bulb of *Scilla campanulata* cut in a longitudinal plane to show the succulent tissues at the base of the scales reduced by *Anguillulina dipsaci*, aided by secondary invaders, into a putrid mass, the worms having previously worked their way downwards along the old floral stalk (see pp. 424, 426, 427).

Figs. 7, 8. Bulbs, essentially similar to that in fig. 6, cut transversely to show that the resistance afforded by the walls of the inner scales, in contrast to the outermost ones (figs. 4, 5), is comparatively small, the nematode being capable of spreading almost as readily in a radial plane (fig. 8) as in a downward direction (figs. 6, 7) (see pp. 424, 426).

Fig. 9. Healthy bulb of *Scilla campanulata*.

Figs. 10–14. Bulbs of *Scilla campanulata* showing reactions differing from those commonly associated with attack by *Anguillulina dipsaci* in Monocotyledons. Note the misshapen and greatly enlarged bulb in fig. 10 compared with normal one in fig. 9, the emergence of a shoot through the badly decayed base-plate in fig. 11, the fissures in the outer scales caused by the increased volume of the underlying tissues in fig. 12, the cracking and decaying having continued still further into the tissues particularly at the apex in fig. 13, and finally, the entire bulb having disintegrated with only the small developing buds remaining in fig. 14 (see pp. 424, 425).

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EDWARDS.—Observations on a Disease of *Scilla campanulata* Ait. due to the Stem and Bulb Eelworm, *Anguillulina dipsaci* Kühn (pp. 422–432)

EDWARDS.—Observations on a Disease of *Scilla campanulata* Ait. due to the Stem and Bulb Eelworm, *Anguillulina dipsaci* Kühn (pp. 422–432)

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

GENERAL MEETING of the Association held at 2.0 p.m. on Friday, 15 March 1940, in the rooms of the Linnean Society at Burlington House, Piccadilly, London, the President, Mr C. T. Gimingham, in the Chair.

Discussion on "Efficiency with economy in the control of plant diseases and pests"

The following papers were read:

I. The general problem and the transition to war conditions. By H. MARTIN.

II. Protective fungicides. By M. H. MOORE.

III. Some entomological aspects of the problem. By S. G. JARY.

IV. Some factors determining the efficiency of spraying operations. By C. DAVIES.

V. Some practical aspects of the spraying of fruit. By A. J. WOOLDRIDGE.

I. THE GENERAL PROBLEM AND THE TRANSITION TO WAR CONDITIONS

By H. MARTIN

Agricultural and Horticultural Research Station, Long Ashton, Bristol

So many and so varied are the devices used to avoid or to control the diseases and pests of crop plants that their number embarrasses a brief review of their relative efficiency and economics. Looking at the problem first under normal peace conditions, the ideal method is the use of immune or adequately resistant varieties. The need for control measures is thereby abolished but the practical difficulties are great. As yet, resistance to but a limited number of parasites has been attained in the most successful applications of this method and the possibility of the evolution of strains of parasites able to attack the immune variety cannot be eliminated. In spite of the difficulties, the principle is so fundamentally sound and is so rich in attractive genetical and biochemical problems that it deserves the fullest attention. It is good to find that, in every country, the application of this principle is being studied; here, for example, against Woolly Aphis at the East Malling Research Station and the John Innes Horticultural Institute.

Then there are the many methods by which the resistance of the host is enhanced or its liability to attack is reduced by cultural means. In this category may be included the modification of nutrient conditions by manuring, the choice of soils or localities where environmental conditions favour the plant and discourage the parasite, cultural management such as the selection of "seed", the control of planting dates, and the sequence of crop rotation. In the distant past these methods were applied in an empirical and perhaps unconscious manner, for they were operative factors in the evolution of the local agricultural practice. More recently scientific attention has been focused on epidemiology and an extension of research of the type initiated by the late Dr Maldwyn Davis on the aphis-vectors of potato viruses will be followed by great advances in Plant Protection. The completion of soil surveys and of the more local surveys of the pest population of the soil will provide a trustworthy scientific basis for agricultural extension and development.

These cultural methods rest more on disease or pest avoidance than on control and they have been strongly advocated in recent years by a school led by Sir Albert Howard, who goes so far as to maintain that other methods of plant protection are unsound. Indeed he suggests that it is a mistake to combat diseases and pests by other means for they are indicators of improper cultivation or selection. The comments of the hop-grower when told that the necessity for aphis control is the reward for his ignorance of hop-growing would be terse but unfit for publication. It is unfortunate that the exaggera-

tion of Sir Albert Howard's claims tends to mask rather than to emphasize the value of cultural methods of plant protection, the importance and possibilities of which are already appreciated by growers and by pathologists.

The third group of control measures may be termed the chemical methods for they involve the use of insecticides and fungicides to combat or to prevent attack on the plant or on the "seed" in the soil, or to prevent the dissemination of the parasite. It is on this group of methods that the present discussion will largely centre, for it is here that research has led to great improvements in efficiency which is the royal road to economy. Once the life history and epidemiology of the pathogen has been elucidated and the feasibility of chemical control established, the main incentive to research has been the efficient use of the chemical. Time permits only a mention here of the more important of these advances. Work on the mechanics of spray application has pointed the way to economies in time and labour. The elucidation of the physico-chemical factors involved in spray retention has provided a sound basis for the development of combined washes with economy in the number of applications. The discovery of the properties of the active constituents of complex products such as the plant insecticides or the hydrocarbon oils has enabled reductions in the recommended concentrations. The synthesis of new insecticides has already enabled the derivation of more efficient and more certain sprays. The practical effect of these developments is illustrated in winter washing. The economy in cost and time of application is shown in figures given by Swarbrick. The winter washing of Plot 7B at Long Ashton took, in the 1928–9 season, 23 hr. at a cost of application of 61*s.* 0*d.* whereas, in 1935–6, the same plot took 6½ hr. to spray at a cost of application of 16*s.* 8*d.* The concentration of tar oil recommended in winter washes has been approximately halved in the same period as a result of better knowledge of the physico-chemical properties determining ovicidal efficiency, and of the limitations of tar oils as ovicides. About 1930 it became apparent that certain grades of petroleum oil would outdo tar oils for the control of capsid bugs, which are no longer feared by the fruit grower. Yet another pest, Red Spider, will cease to cause anxiety to the fruit grower if the results of preliminary trials of tar oil substitutes such as dinitro-*ortho*-cresol and *n*-butyl carbitol thiocyanate in combination with petroleum oils are borne out in practice. The utility of winter washing, so greatly enhanced by the development of the tar oil wash at the end of the last war, will then have been extended to cover all the more important pests assailable in the dormant season.

But again we are at war and thought must be given to the present status of the control measures on the classification outlined above. The aim is clear and simple. No longer is it the production of the most profitable crop, the "fancy" variety which by its pleasing flavour or perfect appearance can secure a high price under peace-time conditions, but it is the greatest possible production of food material per acre. Yield is the war-time criterion by which the efficiency of control measures is to be judged. The immune variety stays only if it is the most certain way to maximum yield. Of the cultural methods the only measure which remains unaffected, at least under present conditions, is that of proper manuring, but possible restrictions in supplies of potash and of nitrogenous fertilizers must be remembered. The selection of soils and localities in which environmental conditions tend to reduce the degree of infestation is now restricted. The ploughing up campaign will bring under cultivation acre after acre of ground on which, in pre-war days, arable crops yielded inadequate profit. The slogan "dig for victory" will encourage the growing of crops and varieties in localities where, under peace conditions, their cultivation was not attempted. The choice of "seed" material will be restricted perhaps to a degree which would necessitate the use of susceptible varieties or of contaminated seed. The peace-time crop rotation may have to be abandoned and a rigid adjustment of the planting or sowing dates may be frustrated by labour factors or the effort to produce the greatest yield.

For these reasons the transition from peace to war conditions will curtail the applicability of cultural methods of pest and disease control and, consequently, greater responsibility will be borne by the chemical methods. In cereal growing, for example, the limitation of supplies of disease-free seed can be countered by an extended use of seed dressings. The expansion of potato growing to localities in which Blight (*Phytophthora infestans*) is prevalent must be followed by the adoption of control measures against this disease. But war conditions, while requiring the extension of chemical methods of plant protection, also bring difficulties, to be overcome if this extension is to be effective and economical. These difficulties may be grouped under the headings of appliances, labour and material.

Present day control measures based on the use of insecticides and fungicides require, in general, special appliances ranging from the elaborate central pumping stations and underground pipes of the modern fruit farm to the simple churn mixer for seed disinfection. But engineering firms are fully occupied with war work and it may not be possible for individual growers to obtain new equipment.

A shortage of appliances and of men skilled in their use is likely but this may be alleviated in various ways, some of which are already in use. The Somerset War Agricultural Executive Committee has recruited growers who are prepared to lend their spray machines to other growers on a contract basis, the primary purpose being to encourage the winter spraying of farm orchards. In Cardiganshire, the corresponding Committee have been asked to purchase suitable seed-dressing machines, which are to be distributed to suitable local authorities from whom they could be hired by farmers. The point arises, in passing, whether it is better to encourage seed dressing by the seed merchant rather than by the grower. The seed merchant often has better storage facilities and the risk of the feeding of treated seed to stock is reduced. It has also been suggested that, in market garden areas, trailer pumps and spray equipment should be provided to encourage the wider use of sprays by growers whose individual acreage is insufficient to justify the purchase of spray machinery. Simultaneously, methods of application might be devised for which no special appliances or skill are necessary. At Long Ashton, for example, the small scale field comparison of the fungicidal efficiency of copper-containing sprays has been facilitated by the simple device of sprinkling the spray over potatoes by means of a watering can fitted with a fine rose. The results have left no doubt of the feasibility of controlling Blight by this means. If a dust is preferred little ingenuity is required to devise a simple container and sieve, such as the muslin bag, from which the dust can be distributed over the plant. The disadvantages of such simplified methods of application are that a greater amount of material is applied per acre than from sprayer and duster nozzle and that the labour requirements are high. Yet these disadvantages count for little when it is remembered that such methods would be recommended only to the small grower for use on low-growing crops, that is to the amateur gardener and allotment holder. Further no skill is required, but merely a little common-sense and some degree of agility, far less than is required for the proper use of the knapsack sprayer which is the appliance at present recommended in the "Growmore" publications.

The sprinkling method has, so far, been tested only for Potato Blight control but the method is applicable to the control of pests and diseases of vegetables and bush fruit. It would facilitate an extension of the commercial methods of controlling pests such as the Raspberry Beetle or diseases such as American Gooseberry Mildew to private gardens where at present the zest of the amateur does not overcome his reluctance to purchase and to use a knapsack or syringe. There seems to be only one condition necessary for success, that the spray should wet the foliage perfectly. In contact sprays such as would be used against the beetle and the mildew mentioned above, this property is obtained by the addition of wetters whereas potato foliage is so easily wetted by water that even Bordeaux mixture may be sprinkled satisfactorily. The general trend of the Long Ashton experiences indicates, however, that the addition of a small amount of wetter is advantageous, which raises the question of materials.

At the present moment, it would appear that the only restriction imposed by war conditions on the supply of pest control materials is due to economic expediency which discourages the importation of compounded products of foreign origin. It would therefore be unwise to count on adequate supplies of materials such as the synthetic thiocyanates from the United States or possibly of the proprietary calcium cyanides. The situation here contrasts strongly with that of Germany and it will be interesting to discover, after the war, what steps have been taken there this time to replace, for example, copper in the protective fungicides. The subject of materials raises, however, a matter of urgent and great importance. If chemical methods of plant protection are to be utilized to the full in the increased production of food-stuffs, every step which simplifies their use must be taken. The simplified methods of application must be accompanied by simple methods of preparing the sprays or dusts. Full use should be made of the compounded powders and pastes which, if to be applied as sprays, require only dilution with water. The recommendation of home-made Burgundy mixture in the Ministry's Growmore Bulletin No. 1 ignores the several compounded copper pastes and powders which are the outcome of the research of the past decade and which have met with success in the field. Yet this same publication recommends derris preparation used in accordance with the maker's instructions. The disadvantage of higher cost over the home-made spray is insignificant for the purchaser concerned requires but small quantities and would not be prepared to buy and store the larger quantities required for substantial saving possible to the established grower. The really serious difficulty is that, as the compounded product is often sold under a trade name, the inexperienced grower has no protection from the inefficient or uneconomical proprietary product. To suggest that the grower should purchase only from reliable firms is useless to the novice unless a yardstick of reliability is provided. Moreover the advisory officer, to whom the grower is recommended to apply in case of difficulty, can only condemn a product or maker at the risk of legal action. The official attitude of ignoring the proprietary product

until sufficient evidence is available for the erection of a minimum specification must therefore be drastically modified if the danger of the inefficient or bogus pest control product is to be eliminated. Under war conditions the loss of crop which exposes the unsatisfactory product cannot be permitted and the adoption of some form of official control over proprietary pest control products should form part of the "Growmore" campaign. In the past the manufacturers have given abundant evidence of their willingness to co-operate, with research and advisory officers, in schemes for the better employment of chemical methods of plant protection and there is little doubt that, at the present time, they would respond wholeheartedly to the Ministry's proposals designed to exclude the unsatisfactory proprietary product.

A final general point concerns the dissemination of advice, for every effort must be used to help the many inexperienced growers recruited to the "Growmore" campaign. The Ministry has worked wonders in the prompt publication of leaflets, bulletins and broadcast messages suitable for these new growers but, with regard to pest control, it may be questioned whether general recommendations on the country-wide basis are, in all cases, preferable to the restricted dissemination of specific recommendations suitable for a given locality. Recommendation for the control of Potato Blight, for example, must be determined by the particular weather conditions of counties rather than by the general climate of the whole country. The machinery for the localized recommendation existed before the war in the chain of provincial advisory biologist, the county agricultural organizer and his horticultural staff. But it would seem, to judge from rumour, that this chain has been broken by the recruitment of the county staff to the services of the County War Agricultural Executive Committees. An authoritative statement of the present operation of extension work and of the status of the Advisory Biologist in relation to these Committees is overdue for the efforts to reach the new grower through the printed words are not enough. They must be supplemented by personal contact and local advice if our present knowledge of disease and pest control is to be utilized with the fullest efficiency and economy.

II. PROTECTIVE FUNGICIDES

(Summary)

By M. H. Moore

East Malling Research Station, Kent

Apple scab is a good example of the large class of diseases that are controlled by protective spraying. Four points are of great importance:

(1) *Correct timing of spray applications.* In a regularly sprayed plantation, the first application should be made for greatest effect just before ripe ascospores are disseminated from dead scabbed leaves of the previous year. In a neglected plantation, scab pustules may be present on the young shoots and buds of susceptible varieties, and the first spray application should be made just after bud-burst. Ascospores are ripe usually about the third week in April, but in some seasons they have been found in the second week, and, in some, not till the fourth week. Apple trees are usually at or near the green-bud stage at this period. Once the spores have caused infection it is much more difficult to check the disease by spraying without risking damage to the tree. If early infection is prevented, later infection is automatically avoided, for dead spores have no progeny!

(2) *Strengths of sprays.* Growth is continuous after bud-burst and frequent cover with fairly weak, safe sprays during the critical early period is better policy than reliance on a strong and possibly harmful one applied very early, even though this may retain its effectiveness longer on the parts sprayed. The weaker spray more frequently applied ensures that *all* new growth is protected as it arises. Provided the weaker spray is applied in advance of infection it is effective; if infection occurs before spraying, the stronger spray is needed to check it and spray damage may result. Some growers regularly apply lime-sulphur at 5 % to apple trees soon after bud-burst. This is wasteful because (*a*) infection at that early stage is very improbable in well-cared-for plantations, (*b*) the leaves are not sufficiently expanded to retain more than a small fraction of spray, (*c*) the spray may damage the buds. Experiment has shown that lime-sulphur at 1 %, applied at green-bud, pink-bud, petal-fall and two weeks later, can give excellent Scab-control, but only if the earliest spray is applied in advance of infection. In commercial practice 2½ or 2 % pre-blossom is quite sufficient if the sprays are correctly timed. A spreader adds nothing to the effectiveness of a protective fungicide, but adds to the cost. It may, however, be helpful when the fungicide is applied after infection has occurred.

(3) *Methods of application.* For sprays to be correctly timed, each application must be made in the shortest possible period. To ensure this over a large area, adequate machinery must be available to keep up a high nozzle output at good pressure so that several men can be fully supplied with sufficient spray to keep them moving. As a result of recent far-reaching changes in spraying methods in this country, such machinery is now available. With high nozzle output of spray, weaker sprays than those hitherto used at low nozzle output are imperative if severe damage to foliage and fruit is to be avoided. Great saving of spray can be effected by the use of instantaneous trigger release valves instead of screw-down taps.

(4) *Adequate and balanced nutrition.* Increasing attention is being paid to the nutritional status of the plant in relation to its susceptibility to disease and to spray damage. There is evidence that manurial treatment has an important bearing on that susceptibility, and that protective spraying can more effectively, more cheaply, and more safely be done where apple trees, though susceptible, are less liable to Scab-infection and spray damage by reason of balanced nutrition. The limited data so far available suggest that an excess of nitrogen promotes susceptibility and that potash acts as a corrective. The effect of humus is uncertain at present, but is being studied.

III. SOME ENTOMOLOGICAL ASPECTS OF THE PROBLEM

(Summary)

By S. G. Jary

South-Eastern Agricultural College, Wye, Kent

At the outset, we may make the assumption that efficiency and economy, in pest control are, if not actually identical, at any rate very closely connected from the practical point of view. There are certain factors which, in the application of control measures, the grower must take into account if a high degree of efficiency is to be attained, but there is also a limit to the expenditure in time and materials, which is worth while. In other words, what we call a "commercial control", represents a compromise of absolute efficiency with economy.

It is convenient, for this purpose, to group crops as follows:

Hops. This crop is considered first, not because of its importance, but because it has probably been subjected to more intensive study than any other. Measures against pests are a regular feature of hop culture and reach a very high standard in commercial practice, frequent applications of nicotine wash being given for Aphis and, where necessary, lime sulphur for Red Spider. Poor crops are comparatively scarce and until the recent introduction of control over the crop, one was not infrequently told that hops were now so easy to grow that they no longer paid—an interesting sidelight upon the effect of concentrated research on the economics of crop production. It also points to the fact that the level of production can be very greatly increased by the application of what may be called, in popular terms, scientific methods.

Fruit. Fruit culture is almost entirely concerned with the woody, perennial types of plants, which have a more or less stable insect and allied fauna associated with them. Throughout the year, therefore, it may be possible to put into operation some type of control measure and the plants lend themselves to chemical methods of control. The application of insecticides, in spray or dust form, is carried out as part of a routine and on apples, which receive the most intensive treatment, the programme may cover 6 months of the year. It normally commences with a tar oil wash in the dormant season, a petroleum oil wash a few weeks later, where Capsid Bug is present, followed by a succession of lime sulphur, lead arsenate and nictotine washes against various pests and diseases. Without considering in detail the spray applications which may be necessary on different types of fruit, one may put forward the following points as being of primary importance in all spray applications.

(*a*) *The intelligent use of insecticides.* The grower should know, apart from certain seasonal exceptions, the pests with which he has to deal. A sound programme should be prepared and carried out; haphazard measures are seldom satisfactory or successful. Some growers still continue to apply washes at various times, in the hope that they are doing some good, rather than for any specific reason, while others do insufficient spraying and suffer in consequence

(*b*) *The correct timing of applications.* This is a factor of particular significance, not only as affecting pest control in many instances, but also in relation to the phytocidal effect of some washes.

(*c*) *The use of correct concentrations and the careful measurement of quantities.* The strengths of washes advised are, in general, adequate to bring about good control of particular pests. Especially in the case of oil washes, they are normally the lowest useful concentrations, so that to decrease them still further is to jeopardize the chances of control while an appreciable increase might result in marked plant injury. A not uncommon tendency is to apply a wash at a concentration somewhat higher than that advised, but the most frequent source of trouble is the inaccuracy of measurements, both of the volume of concentrate and of water for dilution.

Vegetables. Pest attacks are of somewhat the same order of importance on vegetables as on fruit, though, generally speaking, they have received less attention. Vegetables are, however, usually either annual or biennial plants and reseeding or planting can readily be carried out to compensate, in part, for failures. If insecticides are used, they must not be dangerous to human beings nor must they injure foliage. Under glasshouse conditions, the plants are normally subjected to fumigation for the control of pests on the aerial portions, the main factors contributing to success being the air-tightness of the house and the maintenance of the necessary temperature and humidity. The last two factors may have a marked bearing upon the action of particular fumigants. In the open, while Aphides occur commonly and are normally dealt with by applications of nicotine, control is not easy on all crops, the Cabbage Aphis being a case in point. The various Root Flies annually cause serious losses, but attacks vary greatly in intensity from year to year and can only be combated by taking preventive measures, i.e. the grower must assume that attacks will occur and take the necessary precautions. Both calomel and naphthalene dusts have considerable value in this respect but, again, applications must be timed with some accuracy if they are to be effective. One of the most serious aspects of intensive vegetable culture is the widespread incidence of Eelworm attacks, particularly the Potato Eelworm. Here the fundamental principle of rotation as a measure of pest control should be fully stressed.

Agricultural crops. While the major part of research work on pests and diseases has centred round intensively grown crops, it should not be forgotten that general agriculture is practised over a far larger acreage than any other form of culture. Even in Kent, predominantly a "fruit" county, only about 15 % of the agricultural land, excluding rough grazing, is devoted to fruit. Much of the essential food production is associated with the remaining 80 % or so, and pest losses, in the aggregate, must amount to a very considerable figure. That they are less in evidence is largely due to the fact that plant pathologists have so little contact with the great majority of farms and especially with the less progressive farmers. The low acreage return on most agricultural crops makes the use of expensive insecticides almost prohibitive. Paris Green for Leather-jackets, Derris for Flea beetles and for Warble Fly control, almost exhaust the list, though "meta' for Slugs may find a place. The more valuable "seed" crops would repay for applications of insecticides and the drilling of wheat between sugar beet rows is an instance of the type of measure which may be applied in agriculture to the more remunerative crops. In general, however, cheaper measures must be sought and we tend to look to "cultural" control as the farmer's chief defence against pests, despite the fact that the question requires almost more research than any other, in order to elaborate suitable methods. Too few farmers realize, in any case, what can be done in this way and there is a tendency to think that only some "chemical" application can do any good. The practices which result in high yield in agricultural crops are, in themselves, largely instrumental in rendering insignificant the attacks of many pests. The effect of early sowing of spring oats on the incidence of Frit Fly attacks has been well substantiated and quick initial development, associated with the presence of available phosphates, is known to be an important factor in minimizing Gout Fly attacks on barley. In general, too little effort is made to ensure the optimum conditions for these crops. Flea Beetle injury on brassicae is also governed, to some extent, by the nature of the seed bed and, though under some weather conditions attacks may develop in any case, the power of resistance and recovery of the plants is markedly affected by soil conditions. Less evidence exists in the case of that almost omnivorous pest, the Wireworm, but, with adequate attention to cultural conditions, not only may the initial attacks be lessened, but the power of recovery of the plants may also be assisted. Farming for high yields, through sound cultivation and high fertility is, in itself, an insurance against serious loss by pests and that loss, now often severe on the less efficiently managed farms, would automatically be reduced if the general level of farming could be raised.

IV. SOME FACTORS DETERMINING THE EFFICIENCY OF SPRAYING OPERATIONS

(Summary)

By C. Davies

South-Eastern Agricultural College, Wye, Kent

The engineer defines efficiency as the ratio between input and output and he always strives to make this as great as possible. In all spraying operations a machine is used and it should be the aim of whoever is responsible for the control of it to get as large an output of useful work from it as possible. I shall enumerate the main points which should receive attention to ensure that it gives an efficient performance.

There are several factors which contribute towards the efficiency of any spraying problem, such as management, the workmen, the mobility of the machine and its accessaries, the mixing plant and the spraying machine itself. I shall confine myself to the last of these, and briefly discuss the care of the more important parts of the machine.

Motor. Assuming that some form of engine is used, it is a good plan to draw up a simple and concise list of routine jobs to be done before, during and after spraying to keep it in proper order, and to see that the points put down are regularly attended to.

Pump. Whether this is a small syringe or a large power pump regular attention is needed to keep it in efficient working condition. If the plunger has leather washers on it these must be kept clean and pliable. Leather and rubber valves must also be kept free from dirt and lime deposits, and if worn or perished renewed. It is an excellent thing to pump clean water through the pump after every day's work. This washes away deposits, which if allowed to settle and harden might involve hours of delay next time the machine is used. Glands and stuffing boxes should be overhauled and repacked with suitable greasy material when the old packing has worn or hardened. Many knapsack sprayers are fitted with a diaphragm pump, the diaphragm of which is usually made of rubber. This has to be renewed at intervals, generally every second season.

Regulator or relief valve. Make certain that this is operating so as to act as a safety valve as well as a pressure regulator. If fitted with a rubber diaphragm remember that rubber perishes in time, and this part will have to be renewed after some service.

Pressure gauge. This, as a rule, does not give a true record of the pressure of the pump, and the pressure at the nozzles is always less than that shown at the gauge. If desired have the gauge tested against a known accurate one.

Strainers. Keep these thoroughly clean if you would avoid frequent delays due to blocked nozzles, and do not tolerate their removal.

Hose and pipes. See that these are of suitable dimensions and materials to handle the fluids you use. Do not allow rubber hoses to kink and never permit carts, etc. to pass over them. Keep connectors handy for rapid repairs in the field. Underground pipes must be pumped through with clean water every day.

Couplings. Select couplings which can be adjusted in the field rapidly and with few tools. Coarse threads on the screws are desirable.

Taps. For high pressure power-spraying taps of the tapered plug and diaphram pattern are quite unsuitable. Those with a lever, instead of a screw, are generally to be preferred. The valves and glands have to be renewed or adjusted at intervals. Leaking taps are a source of waste and annoyance.

Nozzles and lances. Decide on the type of spray you intend to use, and remember that the design of the eddy- or vortex-chamber mainly controls the fineness, and the disk aperture the quantity which issues from the nozzles. You will also have to consider the "carry" of the spray and the best length of lance to which to attach the nozzle or nozzles: the longer the easier it is to reach high branches, but the more unwieldy it is to handle. Get the "balance" of any spray lance or gun when it is actually discharging liquid at pressure.

Equip each nozzleman with spare parts for the nozzles he uses, and a suitable spanner to effect repairs in the orchard.

The advisability of providing hose-pullers should be considered. This can be a young boy attached

to each nozzleman. Or three men to two lances can be employed; each taking it in turns to pull the hoses for the others. Some growers have experimented with hose harness with some success.

Take all possible steps to prevent stoppages and breakdowns when the work has started.

V. SOME PRACTICAL ASPECTS OF FRUIT SPRAYING

(Summary)

By A. J. Wooldridge

Apart from any thoughts of war, many growers will be seeking to cut down their spraying costs because of the low prices obtained for their produce in 1939, but this year especially it is of the utmost importance to maintain clean orchards: (1) because we may not have the labour available later on to carry out a full programme, (2) prices of insecticides and fungicides may increase considerably, and (3) it is much cheaper to maintain a regular routine than to slack off one year and then have to rectify the trouble at increased cost.

My farm of 76 acres of fruit comprises 31 acres of Bramley's, 30 years old, 40 ft. apart and touching, 15 acres of large, spurred Lord Derby, 2 of Lane's, 1 of Early Victoria, 10 of Cox's Orange Pippin, 5 of plums and 12 of cherries. I use tar-oil at 3 % on all trees, in mid-March on cooking apples, and this is satisfactory. I rely on nicotine (8 oz./100 gall.) in the pink-bud spray for capsid control and use no petroleum because of the risk of spray damage in some seasons. I find this entirely satisfactory, thanks to my present spraying equipment. For scab, I apply lime-sulphur (2½ %) at green- and pink-bud on the cooking apples, and vary the post-blossom treatment (1 or ¾ %) for the variety. Cox's Orange Pippin receive lime-sulphur at 2 % at "mouse-ear" and green-bud, and 1 % at pink-bud. Thereafter I rely on lime-sulphur (½ %) with colloidal sulphur (0·2 %) or colloidal sulphur (0·4 %) alone for them.

It is a wise policy to formulate one's plans for the next year's spraying while the crop is still on the trees.

I regard adequate light and free air movement among the trees as a necessary preliminary to satisfactory fruit quality and quantity; it also saves spraying costs.

While capital expenditure on spray machinery is a heavy item, the additional small outlay required for a slight increase in horse power and pump capacity over the minimum thought desirable is amply repaid, for the work can then easily be speeded up when necessary. A man good with a lance ranks highly with me and, provided his other work is good, he receives a higher wage than the man who, though efficient at other work, is not so with spraying.

In 1935 I used a 4 h.p. machine with portable pipes and single-nozzled, 6 ft. lances, when four men laboriously sprayed 6 acres a day. During the following winter I installed a 13 h.p. Diesel engine and 4-throw 3¼ in. pumps as a central unit operating through underground mains. At first I used the same lances and nozzles but soon changed them for 4 ft. lances and quadruple nozzles after a short period with double nozzles. With these and a pressure of 500 lb. at the pump, three men now average 14 acres a day. The use of trigger valves effects a great saving of wash with this high nozzle output. Renewal of worn disks effects a further saving and is worth the expense.

Where there is a correct balance of fertility in the soil, I think there is possible resistance to fungus attacks, which thus indirectly lowers spraying costs.

To sum up—with sufficient power and pressure it is possible to get a commercial control with a minimum of cost through being able to spray the farm quickly and effectively. Even so, I think that these moderate costs are not attained in one year; deliberate attempts are necessary over several years to get the orchards into a hygienic condition. Once that standard is reached it is the less costly to maintain it.

I am fully aware that I may have to alter the programme, but for the last three years it has given me satisfaction.

REVIEWS

Field Plot Technique. By WARREN H. LEONARD and ANDREW G. CLARK. Pp. 271. Minneapolis, Minn., U.S.A.: Burgess Publishing Co. 1939. $3.25.

The authors of this book are respectively Professors of Agronomy and of Mathematics at Colorado State College; those to whom mathematics is anathema will find comfort in the predominance of agronomy. In their preface the authors state that the book has developed from a course of lectures to graduate students. The style still bears many marks of its class-room ancestry, especially in the note-book terseness of its phraseology and in the naiveté of remarks such as "Some imagination is essential in the research worker but, of course, it must be scientific imagination".

Each of the twenty-one chapters is followed by an extensive list of references, which, if used with discrimination, should prove not the least of the book's merits, and by numerous "questions for discussion". These last are mostly unimaginative, even from the class-room point of view, since their answering is little more than a recapitulation of the preceding chapter. The first four chapters discuss general principles of biological research and the history of agricultural experimentation, with particular reference to the United States. The space allotted is too short to do full justice to such a subject, but the notes given provide an interesting introduction to the main theme.

The second section of the book, describing methods of statistical analysis, is a reasonably sound account of the subject from an elementary standpoint, though the reviewer may be pardoned for doubting whether the authors are themselves always certain of the theoretical background to the tests described. Thus, the suggestion that bimodal distributions may be treated by Pearson or Gram-Charlier methods of curve-fitting requires more justification than a footnote. Again, in explaining the testing of the difference between paired variates, an example suggests that the test to be adopted may be selected as that one giving the most acceptable result, rather than being determined by the design of the experiment. The equivalence of Student's pairing method and the analysis of variance of a $2 \times n$ table is not mentioned.

In their example to illustrate Fisher's test of significance of the difference between two correlation coefficients, the authors fall victims to an error to be found in many recent books on statistical method. The two coefficients whose differences are to be tested are not independent, being obtained from the same set of varieties, and the test is not applicable. Had the emphasis of this chapter been placed on the more fundamental notion of regression, instead of on correlation, this mistake would probably not have arisen.

The third and largest section is devoted to experimental design and details of technique. In addition to discussion of many of the latest developments in design, the reader will find here much interesting information and advice on the conduct of different types of experiment and the difficulties associated with particular crops. It is surprising to find the complex subject of crop-rotation experiments dismissed in half a page. The authors do not always discriminate very satisfactorily between generally accepted practice, personal opinions, and arbitrary rules. In the chapter on sampling methods, for example, the reader is given little assistance in deciding which method is appropriate to his purpose since no comparison of the different techniques is made. The reviewer cannot find any mention of the necessity of samples being selected by a random process in order to give unbiased estimates of the populations sampled. The book is completed by a selection of standard statistical tables.

This volume is a useful contribution to the literature of a rapidly expanding subject, but the reader for whom statistical method still has an appearance of legerdemain must be warned against placing too great a faith in the validity of the more theoretical arguments. On the other hand, the examples, both worked and unworked, are mostly good and worthy of careful study. The mimeographic reproduction, at least in the reviewer's copy, is more legible than usual, but contains many minor misprints. The date of foundation of Rothamsted Experimental Station was neither 1841 (chap. I) nor 1838 (chap. II), but 1843. Question 15 on p. 50 is incorrect as stated, but is sufficiently near the truth to mislead some. D. J. FINNEY.

The Chemistry and Toxicology of Insecticides. By HAROLD H. SHEPARD. Pp. 383. Minneapolis, Minn., U.S.A.: Burgess Publishing Co. 1939. $ 4.00.

The volume of research work carried out on the control of insect pests by chemical means has, during the last twenty years, grown to large dimensions. Not only so, but the subject is by no means a narrowly restricted one, since its range covers many branches of chemistry and a good deal of physics in addition to entomology, in which the physiological side is likely to play an important part in the future. Since also modern toxicological research is becoming increasingly quantitative, statistical methods are more and more employed in insecticidal investigations both in the laboratory and in the field. The literature of the subject has become so extensive as to be almost beyond the range of the average research worker and student, who frequently are without access to a good library containing it, and who need not only a digest of it, but some means of obtaining a co-ordinated view of the subject as a whole.

Professor Shepard, a distinguished worker and teacher in this field, has under the above title published his lecture notes in mimeographed form, and by so doing he has placed both students and research workers on insecticidal problems in his debt, for although there are a number of admirable monographs on specific aspects and one or two good general textbooks, these notes are without question the most complete survey we have of the literature as a whole and they present a co-ordinated review of the many aspects of the subject. Moreover, the bibliographies appended to each section are so extensive in their scope that the reader can find ready access to the original papers cited and to the more specialized reviews.

The author makes little or no attempt to deal with the apparatus and machinery employed in the application of insecticides, although much valuable work has been carried out in recent years on spraying, dusting and fumigation technique, both in the laboratory and in the field. It is, perhaps, rather regrettable so far as laboratory biological methods are concerned, but the omission of a description of field machinery is amply justified, since to have invaded what is primarily the province of the engineer would have distracted attention from the author's main thesis. A brief account, however, of the lay-out and statistical treatment of field spraying experiments would have been welcome.

The first four chapters dealing with the introduction, history, principles and the quantitative technique employed in assessing insecticides only occupy 28 pages, and this paucity of treatment might easily be made a matter for criticism: indeed these very interesting chapters could with advantage have been expanded. Their brevity would seem to be due to the exigency of the lecturer's role, in that practical illustration has to be incorporated in a syllabus of teaching and much fundamental matter which illustrates these earlier chapters is to be found in those which deal with specific subjects such as the stomach poisons, the contact insecticides and fumigation, which are very fully and copiously treated, from chemical, physical, toxicological and physiological points of view.

The five chapters dealing in detail with the several types of insecticides, their application and mode of action are packed tight with the most varied information, giving these notes a high quality as a book of reference, which is enhanced by the long lists of notices of the literature appended to each section. The reading of them is not particularly easy, since there is frequently a certain disjunction between consecutive paragraphs, so that they constitute islands of concentrated fact and data. The arsenical and non-arsenical stomach poisons in their chemistry and toxicology to insects and other animals and the attendant problems of spray injury and residues have detailed attention. The contact insecticidal action of sulphur and the oils are treated in two chapters, in the latter of which much attention is given to the important matter of adjuvants. The problems of wetting, spreading and emulsification are thus given a separate section in the chapter dealing with oils, but so important is the subject that the dignity of a separate chapter would seem to have been indicated as the appropriate treatment.

Plant derivatives and the new synthetic organic compounds are given a chapter by themselves "since they might fall into one or more of the classes of insecticides", but it is not easy to understand why the consideration of the insecticidal properties of the nitrophenols should be in the chapter dealing with the coal tar derivatives, and not with the other synthetics which are worthy of a separate chapter, since they are not necessarily related to the plant products. The plant insecticides are rather briefly treated but on the whole adequately with the exception of the chemistry of the rotenone-containing class, which is not quite up to date. The optically active form of toxicarol has now been known for some time, and, in addition, the two recently isolated compounds elliptone and malaccol have been added to the list of the active principles of leguminous fish-poison plants, but perhaps too late for incorporation. In the pyrethrum section the work carried out at Rothamsted on the loss of activity of pyrethrum on exposure to light and air and means of stabilization is briefly noted, but no mention of

this work and its date is found in the accompanying table of references, nor are the dates and references to the work of Chevalier & Dantony or of Ripert, cited on the same page, readily traceable. These may be mere slips but they detract somewhat from the value of the book as a work of reference. There are a few other omissions of important European papers. For information about the cultivation and harvesting and specific uses of these insecticide plants reference to special monographs or papers would have to be made and one does not find a reference to Potter's important pyrethrum-oil technique used in warehouse spraying.

An error of fact is noted on p. 288; rotenone combines with one molecule of dichloracetic acid and not two of acetic acid as stated. On p. 342 also, Tattersfield *et al.* (1925) (the date is given erroneously as 1935) found naphthalene more toxic to *Aphis rumicis* than *p*-dichlorbenzene as sprays, not as vapours as stated, under conditions in which the fumigation effect would not be clear.

From a minor historical point of view it should, perhaps, be noted that Holt's paper in the *Lancet* seems to have antedated the more important work of Moore in setting out the boiling point theory of toxicity. The book, however, as far as the reviewer can check the large accumulation of information, is comparatively free from errors of fact.

A few unimportant grammatical errors which have escaped the author's notice have been detected here and there throughout the text; they do not detract materially from the book's quality. A useful subject index is included but it could with advantage be enlarged, and for reference purposes the incorporation of an author index would have added greatly to the value of this monograph. It is to be hoped that Professor Shepard will publish these notes in a more permanent form and that readers will respond to his request for suggestions and criticisms. The reviewer has already found them useful for purposes of reference, and they may have to stand up to a good deal of wear and tear in laboratories and libraries possessing them.

F. TATTERSFIELD

An Outline of Diseases of Cereal and Forage Crop Plants of the Northern Part of the United States. By JAMES G. DICKSON. Pp. vii + 259. Minneapolis, Minn., U.S.A.: Burgess Publishing Co. 1939. $ 3.00.

This book contains straightforward accounts of the non-parasitic, virus, bacterial, and fungal diseases of barley, corn, oats, rye, sorghums and millets, wheat, flax, alfalfa and sweet clover, clovers, and soybean. Flax, included under cereals, is a little unexpected but no one will grumble at its inclusion. As its title implies, the book does not claim to be a complete world treatise, but there are only a very few, and those minor diseases of the crops concerned, which are not considered, and the book is the most complete treatise of its kind yet published. Further, the book is not an ordinary textbook, it is as its author describes it an "Outline", and yet so packed is it with data that it forms a first-class source and reference book for advanced students, practising pathologists and research workers. The author assumes in his readers a knowledge of plant physiology and anatomy, plant genetics, fungal morphology, the general principles and methods used in plant pathology, and agronomy. The book is essentially pathological in outlook, subordinating mycological detail to symptomatology, pathogenesis, and control measures.

Each disease is considered according to a more or less standardized schedule and the text is clear, concise, accurate and up to date. Especially valuable is the bringing together in one volume of numerous keys for the separation and identification of physiologic races of smuts and rusts of cereals, and a further notable feature are the bibliographies, although many of the references need more meticulous checking. The book opens with a full Contents, and closes with a "List of bacteria and fungi parasitic on the field crops arranged under orders and families with common hosts", and a good Index. There are no illustrations and the "Mimeograph" process by which the book is produced also reproduces many typing and spelling errors.

The author uses the bacterial nomenclature of E. F. Smith, and throughout adopts the American habit of small letters for all specific names. As American workers differ among themselves in their usage of common names for plant diseases it seems a pity that they cannot adopt our standardized English names where these are available (see *Trans. Brit. mycol. Soc.* List). Also the author might have set a good pattern if he had standardized his method of citing spore measurements.

Owing to a "Federal W.P.A. grant through the University of Wisconsin Natural Science project" the outline is offered "at the cost of publication and distribution. . . and in this preliminary form in the spirit of inviting suggestions and criticisms in relation to its usefulness and contents". Of the general quality of the work one need only say that it is what one expects from a Professor of the Wisconsin School of Plant Pathology.

W. B. BRIERLEY

Diseases of Vegetable Crops. By J. C. WALKER. 2nd edition. Pp. 67. Madison, Wisconsin, U.S.A.: The Author, Department of Plant Pathology, College of Agriculture. 1939. $ 1.75.

A first revision of a book originally published in 1935 (see *Ann. appl. Biol.* 1936, **23**, 918). The text has required little change but new data have been incorporated, and the bibliographies have been improved and brought up to date. Some entire pages are, however, reprinted without change and original mistakes have been repeated: e.g., on p. 26 "mycorrhizal" still appears as "Mychorriza", and on pp. 46–47 the account of wart disease still needs correcting. Owing to the limitations of type possible on a typewriter the headings do not stand out clearly and the book becomes much easier to use if one underlines common names of diseases in colour. I have used both editions of the book with students who find it uncommonly helpful, and I still remain astonished at the amount of information it contains.

W. B. BRIERLEY

The Nematode Parasites of Plants Catalogued under their Hosts. By T. GOODEY. Pp. 80. Imperial Bureau of Agricultural Parasitology (Helminthology), Winches Farm Drive, Hatfield Road, St Albans, England. 1940. 10*s.* 0*d.*

In the first 51 pages the host plants are arranged in column alphabetically by genera and species together with their nematodes, the name of the first recorder, and the date of the record: 28 new records by the author are included. A further section of 11 pages lists over 1000 popular names of plants with their scientific equivalents. The compiling of these lists must have given Dr Goodey many pleasant hours but the use of common names seems to me far too dangerous a practice to receive any recognition in such a publication as this. For example; on p. 52 appears "bachelor's button" and the scientific equivalent is given as *Gomphrena globosa*, but referring merely to three books at hand it took me less than 10 min. to find the names "bachelor's button" and "bachelor's buttons" used indiscriminately for 13 species belonging to 6 genera. *Gomphrena globosa* was not included among these but in one of the books the English common names of this species were given as "globe amaranth", and "red globe everlasting" and only its Italian common name was "battone di baccelliere". The work concludes with two pages of synonyms of scientific names, and 474 references to host and parasite records cited in the text. The compiling and sifting of the literature must have been a labour of love but it has been done with meticulous accuracy and the result is an extremely useful pamphlet. It is the kind of production which ought to be kept up to date by the frequent issue of new editions, but its cost renders this impossible. The cost might have been materially reduced by the frequent use of "ditto" symbols.

W. B. BRIERLEY

Vegetable Crops. By H. C. THOMPSON. 3rd edition. Pp. xi + 578. New York & London: McGraw-Hill Book Co. 1939. 33*s.* 0*d.*

This well-known treatise, now in its third edition, remains obstinately and narrowly United States in outlook: U.S. crops and varieties; U.S. systems of classification; U.S. conditions; U.S. soils, manures, and cultural methods; U.S. storage and marketing arrangements; U.S. plant diseases and pests. Finally, the author lists "the references mentioned in the text" and of the 452 citations all but nine are to U.S. publications! Of these nine, one is to a Polish paper; four are British and, of these, two are to recent semi-popular accounts of boron deficiency, one is to a 1921 semi-popular account of artificial farm-yard manure, and the fourth is to David Livingstone's *Travels and Research in South Africa*, published in 1858: the remaining four are Canadian, two of them being to popular bulletins on potatoes, dated 1905 and 1916 respectively.

The book contains a tremendous amount of useful material but a great deal of the text is wide open to major or minor criticism. Thus there is very little appreciation of the importance of genetics in relation to vegetable crops; the accounts of the history and taxonomy of the several crops seem mostly to be taken from *Sturtevant's Notes on Edible Plants* edited by Hedrick in 1919 and make no mention of the radically different views put forward during the last 20 years; the control measures recommended for plant diseases and pests are not always up-to-date; many wrong spellings of fungal and bacterial names are carried over from the second edition, etc. In spite of these things the book remains exceedingly useful, but the pity of it is that the book is so good that it might so easily have been a great deal better.

W. B. BRIERLEY